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(71) Applicant (*for all designated States except US*): JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): CONTRERAS, Roland, Henri [BE/BE]; University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent (BE). NELISSEN, Bart [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). DE BACKER, Marianne, Denise [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). LUYTEN, Walter, Herman, Maria, Louis [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 beerse (BE). VIAENE, Jasmine, Elza [BE/BE]; University of Gent, K.L. Ledeganck Straat 35, B-9000 Gent (BE). LOGGHE, Marc, George [BE/BE]; University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent (BE). VIALARD, Jorge, Edwards [CA/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE).

(74) Agent: BOULT WADE TENNANT; 27 Furnival Street, London EC4A 1PQ (GB).

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(54) Title: DRUG TARGETS IN *CANDIDA ALBICANS*

(57) Abstract

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides which are critical for growth of *Candida albicans*. Methods for the identification of anti-fungal compounds which inhibit fungal or yeast growth are also contemplated.

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DRUG TARGETS IN *CANDIDA ALBICANS*

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides which are critical for growth of *Candida albicans*.

Opportunistic infections in immunocompromised hosts represent an increasingly common cause of mortality and morbidity. *Candida* species are among the most commonly identified fungal pathogens associated with such opportunistic infections, with *Candida albicans* being the most common species. Such fungal infections are thus problematical in, for example, AIDS populations in addition to normal healthy women where *Candida albicans* yeasts represent the most common cause of vulvovaginitis.

Although compounds do exist for treating such disorders, such as, amphotericin, these drugs are generally limited in their treatment because of their toxicity and side effects. Therefore, there exists a need for new compounds which may be used to treat *Candida* associated infections in addition to compounds which are selective in their action against *Candida albicans*.

Classical approaches for identifying anti-fungal compounds have relied almost exclusively on inhibition of fungal or yeast growth as an endpoint. Libraries of natural products, semi-synthetic, or synthetic chemicals are screened for their ability to kill or arrest growth of the target pathogen or a related nonpathogenic model organism. These tests are

cumbersome and provide no information about a compound's mechanism of action. The promising lead compounds that emerge from such screens must then be tested for possible host-toxicity and detailed mechanism of action studies must subsequently be conducted to identify the affected molecular target.

The present inventors have now identified a range of nucleic acid sequences from *Candida albicans* which encode polypeptides which are critical for its survival and growth. These sequences represent novel targets which can be incorporated into an assay to selectively identify compounds capable of inhibiting expression of such polypeptides and their potential use in alleviating diseases or conditions associated with *Candida albicans* infection.

Therefore, according to a first aspect of the invention there is provided a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in any of Sequence ID Nos. 1 to 9.

Whilst the molecules defined herein have been established as being critical for growth and metabolism of Candida albicans, for some of the molecules no apparent functionality has been assigned by virtue of the fact that no functionally related sequences in other prokaryotic or eukaryotic organism can be found in respective databases. Thus, advantageously these sequences may be species specific in which case they may be used be used as selective targets for treatment of diseases mediated by *Candida Albicans* infection. Thus, in one aspect of the invention the nucleic acid molecules preferably comprise the sequences identified in sequence ID Nos. 1, 4, 5 to 9.

In another aspect of the invention the sequences have been arranged functionally and of nucleotides illustrated in Sequence ID Nos. 2 or 3 are preferred and even more preferably in Sequence ID No. 2 and fragments or derivatives of said nucleic acid molecules.

Letters utilised in the sequences according to the invention which are not recognisable as letters of the genetic code signify a position in the nucleic acid sequence where one or more of bases A, G, C or T can occupy the nucleotide position. Representative letters used to identify the range of bases which can be used are as follows:

M: A or C
R: A or G
W: A or T
S: C or G
Y: C or T
K: G or T
V: A or C or G
H: A or C or T
D: A or G or T
B: C or G or T
N: G or A or T or C

In one embodiment of each of the above identified aspects of the invention the nucleic acid may comprise a mRNA molecule or alternatively a DNA and preferably a cDNA molecule.

Also provided by the present invention is a nucleic acid molecule capable of hybridising to the nucleic acid molecules illustrated in any of Figures 1 to 9 under high stringency conditions such as antisense molecule and which conditions are generally known to those of skill in the art.

Stringency of hybridisation as used herein refers

to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting temperature (Tm) of the hybrids. Tm can be approximated by the formula:

$$81.5^{\circ}C+16.6(Log_{10}[Na^{+}]+0.41\ (\%G\&C)-600L/L$$

wherein L is the length of the hybrids in nucleotides. Tm decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

The term "stringency" refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyramidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency conditions disfavour non-homologous base pairing.

"Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium

chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na_2HPO_4 and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences illustrated in any of Figures 1 to 9.

The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express polypeptides encoded therefrom in a suitable host which are critical for growth and survival of *Candida albicans*.

An expression vector according to the invention includes a vector having a nucleic acid according to the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed, transfected or infected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression

of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

Polynucleotides according to the invention may be inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence given regarding base variations.

The present invention also comprises within its scope proteins or polypeptides expressed by the nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to the invention and preferably from 10 to approximately 120 nucleotides. In another aspect of the invention, nucleotide acid sequences are provided from 10 to 50 nucleotides. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex formation between the probe and any nucleic acid in the sample.

According to the present invention, these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesized *in situ* on the array. See Lockhart *et al.*, Nature Biotechnology, Vol. 14, December 1996, "Expression monitoring by hybridization to high-density oligonucleotide arrays." A single array can contain more than up to more than a million different probes in discrete locations.

Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be between approximately 10 to 120 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA

from a cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolated the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook *et al* (Molecular Cloning: a Laboratory Manual, 1989).

The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{39}S, enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and may be detected using known techniques *per se*.

The polypeptide or protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are substantially homologous to said polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% amino acid homology with the polypeptides encoded by the nucleic acid molecules according to the invention.

Nucleic acids and polypeptides which are particularly preferred are those comprising the sequences of nucleotides illustrated in figures 1 to 3 and polypeptides illustrated in figures 14 to 16. However, a particularly preferred nucleic acid comprises the sequences of nucleotides illustrated in Figures 2 and/or 3, and their corresponding amino acid sequences identified in Figures 15 and 16.

Nucleotide sequences according to the invention

are particularly advantageous as selective therapeutic targets for treating *Candida albicans* associated infections. For example, an antisense nucleic acid capable of binding to the nucleic acid sequence illustrated in any of Figures 1 to 9 may be used to selectively inhibit expression of the corresponding polypeptides, leading to impaired growth of the *Candida albicans* with reductions of associated illnesses or diseases.

The nucleic acid molecule or the polypeptide according to the invention may be used as a medicament, or in the preparation of a medicament, for treating diseases or conditions associated with *Candida albicans* infection.

Advantageously, the nucleic acid molecule or the polypeptide according to the invention may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The present invention is further directed to inhibiting expression of nucleic acids according to the invention *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5′ coding portion or the mature protein sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee *et al.* Nucl. Acids Res., 6:3073 (1979); Cooney *et al.*, Science, 241:456 (1988); and Dervan *et al.*, Science, 251: 1360 (1991), thereby preventing transcription and the production of the

corresponding protein. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the corresponding protein (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse, with the polypeptide according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497.

Antibodies according to the invention may also be used in a method of detecting for the presence of a polypeptide according to the invention, which method comprises reacting the antibody with a sample and identifying any protein bound to said antibody. A kit may also be provided for performing said method which comprises an antibody according to the invention and means for reacting the antibody with said sample.

Proteins which interact with the polypeptide of the invention may be identified by investigating protein-protein interactions using the two-hybrid vector system first proposed by Chien *et al.* (1991).

This technique is based on functional reconstitution *in vivo* of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA

sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a reporter molecule in a GAL4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as ß-galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

Further provided by the present invention is one

or more *Candida albicans* cells comprising an induced mutation in the DNA sequence encoding the polypeptide according to the invention.

A further aspect of the invention provides a method of identifying compounds which selectively inhibit or interfere with the expression, the functionality of polypeptides expressed from the nucleotides sequences illustrated in any of Figures 1 to 9 or the metabolic pathways in which these polypeptides are involved and which are critical for growth and survival of *Candida albicans*, which method comprises (a) contacting a compound to be tested with one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to the invention which mutation results in overexpression or underexpression of said polypeptides in addition to one or more wild type *Candida* cells, (b) monitoring the growth and/or activity of said mutated cell compared to said wild type wherein differential growth or activity of said one or more mutated *Candida* cells provides an indication of selective action of said compound on said polypeptide or another polypeptide in the same or a parallel pathway.

Compounds identifiable or identified using the method according to the invention, may advantageously be used as a medicament, or in the preparation of a medicament to treat diseases or conditions associated with *Candida albicans* infection. These compounds may also advantageously be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

A further aspect of the invention provides a method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival, which method comprises (a) preparing a cDNA or genomic library from

said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library, (b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. Preferably, the cell or organism may be any yeast or filamentous fungus, such as, for example, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

A further aspect of the invention provides a pharmaceutical composition comprising any of a compound, an antisense molecule or an antibody according to the invention together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The antisense molecules or indeed the compounds identified as agonists or antagonists of the nucleic acids or polypeptides according to the invention may be used in the form of a pharmaceutical composition, which may be prepared according to procedures well known in the art. Preferred compositions include a pharmaceutically acceptable vehicle or diluent or excipient, such as for example, a physiological saline solution. Other pharmaceutically acceptable carriers including other non-toxic salts, sterile water or the like may also be used. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for subsequent administration. Incorporation of the polypeptides of the invention into a solid or semi-solid biologically compatible matrix may be carried out which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like. Pharmaceutically acceptable excipients which permit sustained or delayed release following administration may also be included.

The polypeptides, the nucleic acid molecules or compounds according to the invention may be administered orally. In this embodiment they may be encapsulated and combined with suitable carriers in solid dosage forms which would be well known to those skilled in the art.

As would be well known to those of skill in the art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent upon the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner, based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the composition to be administered, the age, weight, and response of the individual patient and the chosen route of administration.

The present invention may be more clearly understood with reference to the accompanying example, which is purely exemplary, with reference to the accompanying drawings, wherein

Figures 1 and 2: are nucleotide sequences isolated from *Candida albicans* and which have an identified function based on sequence homology with proteins from other organisms and which sequences are not present in the public domain.

Figures 3 : is a nucleotide sequence isolated from *Candida albicans* and which has an identified function based on sequence homology with proteins from other organisms and which sequence is partially present in the public domain.

Figures 4 : is a nucleotide sequence of previously unknown function isolated from *Candida albicans* and which is partially present in the public domain.

Figures 5 to 9 : are nucleotide sequences of previously unknown function isolated from *Candida albicans*.

Figure 10 : is a diagrammatic representation of plasmid pGAL1PNiST-1.

Figure 11 : is a nucleotide sequence of plasmid pGAL1PNiST-1 of Figure 10.

Figure 12 : is a diagrammatic representation of plasmid pGAL1PSiST-1.

Figure 13 : is a nucleotide sequence of plasmid pGAL1PSiST-1 of Figure 12.

Figures 14 to 20: are amino acid sequences of the appropriately corresponding DNA sequences illustrated in Figures 1 to 9 with reference to Table 1.

Figures 21 to 27: are growth curves of *Candida albicans* strains showing antisense

induced reduction in growth.

Figures 28 to 31: are growth curves of *Candida albicans* strains including knock-outs in the relevant gene identified.

Example 1

Identification of novel drug targets in *C. albicans* by anti-sense and disruptive integration

The principle of the approach is based on the fact that when a particular *C. albicans* mRNA is inhibited by producing the complementary anti-sense RNA, the corresponding protein will decrease. If this protein is critical for growth or survival, the cell producing the anti-sense RNA will grow more slowly or will die.

Since anti-sense inhibition occurs at mRNA level, the gene copy number is irrelevant, thus allowing applications of the strategy even in diploid organisms.

Anti-sense RNA is endogenously produced from an integrative or episomal plasmid with an inducible promoter; induction of the promoter leads to the production of an RNA encoded by the insert of the plasmid. This insert will differ from one plasmid to another in the library. The inserts will be derived from genomic DNA fragments or from cDNA to cover-to the extent possible- the entire genome.

The vector is a proprietary vector allowing integration by homologous recombination at either the homologous insert or promoter sequence in the *Candida* genome. After introducing plasmids from cDNA or genomic libraries into *C. albicans*, transformants are screened for impaired growth after promoter (& thus anti-sense) induction in the presence of lithium

acetate. Lithium acetate prolongs the G1 phase and thus allows anti-sense to act during a prolonged period of time during the cell cycle. Transformants which show impaired growth in both induced and non-induced media, thus showing a growth defect due to integrative disruption, are selected as well.

Transformants showing impaired growth are supposed to contain plasmids which produce anti-sense RNA to mRNAs critical for growth or survival. Growth is monitored by measuring growth-curves over a period of time in a device (Bioscreen Analyzer, Labsystems) which allows simultaneous measurement of growth-curves of 200 transformants.

Subsequently plasmids can be recovered from the transformants and the sequence of their inserts determined, thus revealing which mRNA they inhibit. In order to be able to recover the genomic or cDNA insert which has integrated into the *Candida* genome, genomic DNA is isolated, cut with an enzyme which cuts only once into the library vector (and estimated approx. every 4096 bp in the genome) and religated. PCR with primers flanking the insert will yield (partial) genomic or cDNA inserts as PCR fragments which can directly be sequenced. This PCR analysis (on ligation reaction) will also show us how many integrations occurred. Alternatively the ligation reaction is transformed to E. coli and PCR analysis is performed on colonies or on plasmid DNA derived thereof.

This method is employed for a genome-wide search for novel *C. albicans* genes which are important for growth or survival.

<u>**Materials & Methods**</u>

Construction of pGal1PNiST-1

The backbone of the pGAL1PNiST-1 vector (integrative anti-sense *Sfi*I-*Not*I vector) is

pGEM11Zf(+) (Promega Inc.). First, the CaMAL2 *Eco*RI/*Sal*I promoter fragment from pDBV50 (D.H. Brown *et al.* 1996) was ligated into *Eco*RI/*Sal*I-opened pGEM11Zf(+) resulting in the intermediate construct pGEMMAL2P-1. Into the latter (*Msc*I/CIP) the CaURA3 selection marker was cloned as a *Eco*47III/*Xmn*I fragment derived from pRM2. The resulting pGEMMAL2P-2 vector was *Not*I/*Hin*dIII opened in order to accept the *Not*I-stuffer-*Sfi*I cassette from pPCK1NiSCYCT-1 (*Eag*I/*Hin*dIII fragment): pMAL2PNiST-1. Finally, the plasmid pGAL1PNiST-1 was constructed by exchanging the *Sal*I/*Ecl*136II MAL2 promoter in pMAL2PNiST-1 by the *Xho*I/*Sma*I GAL1 promoter fragment derived from pRM2GAL1P.

Construction of pGal1PSiST-1

The vector pGAL1PSiST-1 was created for cloning the small genomic DNA fragments (flanked by *Sfi*I sites) behind the GAL1 promoter. The only difference with pGAL1PNiST-1 is that the hIFNβ (stuffer fragment) insert fragment in pGAL1PSiST-1 is flanked by two *Sfi*I sites in stead of a *Sfi*I and a *Not*I site as in pGAL1PNiST-1. To construct pGAL1PSiST-1 the *Eco*RI-*Hind*III fragment, containing hIFNβ flanked by a *Sfi*I and a *Not*I site, of pMAL2pHiET-3 (unpublished) was exchanged by the *Eco*RI-*Hind*III fragment, containing hIFNβ flanked by two *Sfi*I sites, from YCp50S-S (an *E. coli / S. cerevisiae* shuttle vector derived from the plasmid YCp50, which is deposited in the ATCC collection (number 37419; Thrash *et al.*, 1985); an *Eco*RI-*Hind*III fragment, containing the gene hIFNβ, which is flanked by two *Sfi*I sites, was inserted in YCp50, creating YCp50S-S), resulting into plasmid pMAL2PSiST-1. The *MAL2* promoter from pMAL2PSiST-1 (by a *Nae*I-*bal*I digest) was further replaced by the *GAL1* promoter from pGAL1PNiST-1 (via a *Xho*I-*FSPI* digest),

creating the vector pGAL1PSiST-1.

***Candida albicans* genomic library**

* *Preparation of the genomic DNA fragments*

A *Candida albicans* genomic DNA library with small DNA fragments (400 to 1,000 bp) was prepared. Genomic DNA of *Candida albicans* B2630 was isolated following a modified protocol of Blin and Stafford (1976). The quality of the isolated genomic DNA was checked by gel electrophoresis. Undigested DNA was located on the gel above the marker band of 26,282 bp. A little smear, caused by fragmentation of the DNA, was present. To obtain enrichment for genomic DNA fragments of the desired size, the genomic DNA was partially digested. Several restriction enzymes (*Alu*I, *Hae*III and *Rsa*I; all creating blunt ends) were tried out. The appropriate digest conditions have been determined by titration of the enzyme. Enrichment of small DNA fragments was obtained with 70 units of *Alu*I on 10 µg of genomic DNA for 20 min. T4 DNA polymerase (Boehringer) and dNTPs (Boehringer) were added to polish the DNA ends. After extraction with phenol-chloroform the digest was size-fractionated on an agarose gel. The genomic DNA fragments with a length of 500 to 1,250 bp were eluted from the gel by centrifugal filtration (Zhu *et al.*, 1985). *Sfi*I adaptors (5' GTTGGCCTTTT) or (5' AGGCCAAC) were attached to the DNA ends (blunt) to facilitate cloning of the fragments into the vector. Therefore, a 8-mer and 11-mer oligonucleotide (comprising the *Sfi*I site) were kinatesed and annealed. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The DNA fragments of 400 to 1150 bp were eluted from the gel by centrifugal filtration.

* *Preparation of the pGAL1PSiST-1 vector fragment*

The small genomic DNA fragments were cloned after the GAL1 promoter in the vector pGAL1PSIST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with *Sfi*I and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu *et al.*, 1985). Ligation with a control DNA fragment, flanked by *Sfi*I sites, was performed as a control. The ligation mix was electroporated to MC1061 *E. coli* cells. Plasmid DNA of 24 clones was analyzed. In all cases the control fragment was inserted in the pGAL1PSiST-1 vector fragment.

* *Upscaling*

All genomic DNA fragments (450 ng) were ligated into the pGAL1PSiST-1 vector (20 ng). After electroporation at 2500V, 40µF circa 400,000 clones were obtained. These clones were pooled into three groups and stored as glycerol slants. Also Qiagen-purified DNA was prepared from these clones. A clone analysis showed an average insert length of 600 bp and a percentage of 91 for clones with an insert. The size of the library corresponds to 5 times the diploid genome. The genomic DNA inserts are sense or anti-sense orientated in the vector.

***Candida albicans* cDNA library**

Total RNA was extracted from *Candida albicans* B2630 grown on respectively minimal (SD) and rich (YPD) medium as described by Chirgwin *et al.* in Sambrook *et al* 1996. mRNA was prepared from total RNA using the Invitrogen Fast Track procedure.

First strand cDNA is synthesised with the Superscript Reverse Transcriptase (BRL) and with an oligo dT-*Not*I Primer adapter. After second strand synthesis, cDNA is polished with Klenow enzyme and purified over a Sephacryl S-400 spun column. Phosphorylated *Sfi*I adapters are then ligated to the

cDNA, followed by digestion with the *Not*I restriction enzyme. The *Sfi*I/*Not*I cDNA is then purified and sized on a Biogel column A150M.

First fraction contains approximately 38,720 clones by transformation, the second fraction only 1540 clones. Clone analysis:
Fr. I: 22/24 inserts, 16 ^{3} 1000 bp, 4 ^{3} 2000 bp, average size: 1500 bp.
Fr. II: 9/12 inserts, 3 ^{3} 1000 bp, average size: 960 bp cDNA was ligated in a *Not*I/*Sfi*I opened pGAL1PNiST-1 vector (anti-sense)

Candida transformation

The host strain used for transformation is a *C. albicans* ura3 mutant, CAI-4, which contains a deletion in orotidine-5'-phosphate decarboxylase and was obtained from William Fonzi, Georgetown University (Fonzi and Irwin). CAI-4 was transformed with the above described cDNA library or genomic library using the *Pichia* spheroplast module (Invitrogen). Resulting transformants were plated on minimal medium supplemented with glucose (SD, 0.67% or 1.34% Yeast Nitrogen base w/o amino acids + 2% glucose) plates and incubated for 2-3 days at 30°C.

Screening for mutants

Starter cultures were set up by inoculating each colony in 1 ml SD medium and incubating overnight at 30°C and 300 rpm. Cell densities were determined using a Coulter counter (Coulter Z1; Coulter electronics limited). 250.000 cells/ml were inoculated in 1 ml SD medium and cultures were incubated for 24 hours at 30°C and 300 rpm. Cultures were washed in minimal medium without glucose (S) and the pellet resuspended in 650 µl S medium. 8 µl of this culture is used for inoculating 400 µl cultures in a Honeywell-100 plate

(Bioscreen analyzer; Labsystems). Each transformant was grown during three days in S medium containing LiAc; pH 6.0, with 2% glucose/2% maltose or 2% galactose/2% maltose respectively while shaking every 3 minutes for 20 seconds. Optical densities were measured every hour during three consecutive days and growth curves were generated (Bioscreen analyzer; Labsystems).

Growth curves of transformants grown in respectively anti-sense non-inducing (glucose/maltose) and inducing (galactose/maltose) medium are compared and those transformants showing impaired growth upon anti-sense induction are selected for further analysis. Transformants showing impaired growth by virtue of integration into a critical gene are also selected.

Isolation of genomic or cDNA inserts

Putatively interesting transformants are grown in 1.5 ml SD overnight and genomic DNA is isolated using the Nucleon MI Yeast kit (Clontech). Concentration of genomic DNA is estimated by analyzing a sample on an agarose gel.

20 ng of genomic DNA is digested for three hours with an enzyme that cuts uniquely in the library vector (SacI for the genomic library; PstI for the cDNA library) and treated with RNAse. Samples are phenol/chloroform extracted and precipitated using NaOAc/ethanol.

The resulting pellet is resuspended in 500 µl ligation mixture (1 x ligation buffer and 4 units of T4 DNA ligase; both from Boehringer) and incubated overnight at 16°C.

After denaturation (20 min 65°C), purification (phenol/chloroform extraction) and precipitation (NaOAc/ethanol) the pellet is resuspended in 10 µl MilliQ (Millipore) water.

PCR analysis

Inverse PCR is performed on 1 µl of the precipitated ligation reaction using library-vector specific primers (oligo23 5' TGC-AGC-TCG-ACC-TCG-ACT-G 3' and oligo25 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' for the genomic library; 3pGALNistPCR primer :5'TGAGCAGCTCGCCGTCGCGC 3' and 5pGALNistPCR primer: 5'GAGTTATACCCTGCAGCTCGAC 3' for the cDNA library; both from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 57 °C, and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 µM of each primer, 3 mM MgCl2 (Perkin Elmer Cetus) and 200 µM dNTPs (Perkin Elmer Cetus). PCR was performed in a Robocycler (Stratagene).

Sequence determination

Resulting PCR products were purified using PCR purification kit (Qiagen) and were quantified by comparison of band intensity on EtBr stained agarose gel with the intensity of DNA marker bands. The amount of PCR product (expressed in ng) used in the sequencing reaction is calculated as the length of the PCR product in basepairs divided by 10. Sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the instructions of the manufacturer (PE Applied Biosystems, Foster City, CA) except for the following modifications.

The total reaction volume was reduced to 15 µl. Reaction volume of individual reagents were changed accordingly. 6.0 µl Terminator Ready Reaction Mix was replaced by a mixture of 3.0 µl Terminator Ready Reaction Mix + 3.0 µl Half Term (GENPAK Limited,

Brighton, UK). After cycle sequencing, reaction mixtures were purified over Sephadex G50 columns prepared on Multiscreen HV opaque microtiter plates (Millipore, Molsheim, Fr) and were dried in a speedVac. Reaction products were resuspended in 3 μl loading buffer. Following denaturation for 2 min at 95°C, 1 μl of sample was applied on a 5% Long Ranger Gel (36 cm well-to-read) prepared from Singel Packs according to the supplier's instructions (FMC BioProducts, Rockland, ME). Samples were run for 7 hours 2X run on a ABI 377XL DNA sequencer. Data collection version 2.0 and Sequence analysis version 3.0 (for basecalling) software packages are from PE Applied Biosystems. Resulting sequence text files were copied onto a server for further analysis.

Sequence analysis

Nucleotide sequences were imported in the VectorNTI software package (InforMax Inc, North Bethesda, MD, USA), and the vector and insert regions of the sequences were identified. Sequence similarity searches against public and commercial sequence databases were performed with the BLAST software package (Altschul *et al.*, 1990) version 1.4. Both the original nucleotide sequence and the six-frame conceptual translations of the insert region were used as query sequences. The used public databases were the EMBL nucleotide sequence database (Stoesser *et al.*, 1998), the SWISS-PROT protein sequence database and its supplement TrEMBL (Bairoch and Apweiler, 1998), and the ALCES *Candida albicans* sequence database (Stanford University, University of Minnesota). The commercial sequence databases used were the LifeSeq® human and PathoSeqÔ microbial genomic databases (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA), and the GENESEQ patent sequence database (Derwent, London,

UK). Three major results were obtained on the basis of the sequence similarity searches: function, novelty, and specificity. A putative function was deduced on the basis of the similarity with sequences with a known function, the novelty was based on the absence or presence of the sequences in public databases, and the specificity was based on the similarity with vertebrate homologues.

Methods

Blastx of the nucleic acid sequences against the appropriate protein databases: Swiss-Prot for clones of which the complete sequence is present in the public domain, and paorfp (PathoSeq™) for clones of which the complete sequences is not present in the public domain.

The protein to which the translated nucleic acid sequence corresponds to is used as a starting point. The differences between this protein and our translated nucleic acid sequences are marked with a double line and annotated above the protein sequence. The following symbols are used:

a one-letter amino acid code or the ambiguity code X is used if our translated nucleic acid sequence has another amino acid on a certain position,

the stop codon sign * is used if our translated nucleic acid sequence has a stop codon on a certain position,

The letters fs (frame shift) are used if a frame shift occurs in our translated nucleic acid sequence, and another reading frame is used,

the words ambiguity or ambiguities are used if a part of our translated nucleic acid sequence is present in the proteins, but not visible in the alignments of the blast results,

The phrase "missing sequence" is used if the translated nucleic acid sequence does not comprise

that part of the protein.

Blastx: compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database.

Gene Knock-outs

To verify that the growth effect was due to the interference with the identified gene and to support the specificity of the antisense effect, single allele knock-outs were made in the identified genes (Figures 28 to 31) using the URA-blaster method (Fonzi and Irwin 1993).

Screening for compounds modulating expression of polypeptides critical for growth and survival of *C. albicans*

The method proposed is based on observations (Sandbaken *et al.*, 1990; Hinnebusch and Liebman 1991; Ribogene PCT WO 95/11969, 1995) suggesting that underexpression or overexpression of any component of a process (e.g. translation) could lead to altered sensitivity to an inhibitor of a relevant step in that process. Such an inhibitor should be more potent against a cell limited by a deficiency in the macromolecule catalyzing that step and/or less potent against a cell containing an excess of that macromolecule, as compared to the wild type (WT) cell.

Mutant yeast strains, for example, have shown that some steps of translation are sensitive to the stoichiometry of macromolecules involved. (Sandbaken *et al.* 1996). Such strains are more sensitive to compounds which specifically perturb translation (by acting on a component that participates in translation) but are equally sensitive to compounds with other mechanisms of action.

This method thus not only provides a means to

identify whether a test compound perturbs a certain process but also an indication of the site at which it exerts its effect. The component which is present in altered form or amount in a cell whose growth is affected by a test compound is potentially the site of action of the test compound.

The assay to be set up involves measurement of growth of an isogenic strain which has been modified only in a certain specific allele, relative to a wild type (WT) *C. albicans* strain, in the presence of R-compounds. Strains can be ones in which the expression of a specific essential protein is impaired upon induction of anti-sense or strains which carry disruptions in an essential gene. An *in silico* approach to finding novel essential genes in *C. albicans* will be performed. A number of essential genes identified in this way will be disrupted (in one allele) and the resulting strains can be used for comparative growth screening.

Assay for High Throughput screening for drugs

35 µl minimal medium (S medium + 2% galactose + 2% maltose) is transferred in a transparent flat-bottomed 96 well plate using an automated pipetting system (Multidrop, Labsystems). A 96-channel pipettor (Hydra, Robbins Scientific) transfers 2.5 µl of R-compound at 10^{-3} M in DMSO from a stock plate into the assay plate.

The selected C. *albicans* strains (mutant and parent (CAI-4) strain) are stored as glycerol stocks (15%) at -70°C. The strains are streaked out on selective plates (SD medium) and incubated for two days at 30°C. For the parent strain, CAI-4, the medium is always supplemented with 20 µg/ml uridine. A single colony is scooped up and resuspended in 1 ml minimal medium (S medium + 2% galactose + 2% maltose). Cells

are incubated at 30°C for 8 hours while shaking at 250 rpm. A 10 ml culture is inoculated at 250.000 cells/ml. Cultures are incubated at 30°C for 24 hours while shaking at 250 rpm. Cells are counted in Coulter counter and the final culture (S medium + 2% galactose + 2% maltose) is inoculated at 20.000 to 50.000 cells/ml. Cultures are grown at 30°C while shaking at 250 rpm until a final OD of 0.24 (+/- 0.04) 600nM is reached.

200 µl of this yeast suspension is added to all wells of MW96 plates containing R-compounds in a 450 (or 250) µl total volume. MW96 plates are incubated (static) at 30°C for 48 hours.

Optical densities are measured after 48 hours.

Test growth is expressed as a percentage of positive control growth for both mutant (x) and wild type (y) strains. The ratio (x/y) of these derived variables is calculated.

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<u>**Table 1**</u>

Seq ID No.	Figure No.	Clone	Function
1	1	382c_cp	-
2	2	392c_cp	TUF1
3	3	-	RAD53
4	4	417c_cpG2L	-
5	5	323c_af	-
6	6	322c_cp[1]	-
7	7	26g3	-
8	8	409c_cp	-
9	9	382c_cpG1L2	-
10	14	382c_cp (prt)	-
11	15	392c_cp (prt)	TUF1
12	16		RAD53
13	17	325c_af (prt)[2]	-
14	18	322c_cp (prt)[2]	-
15	19	26g3 (prt)	-
16	20	417c_cp 92L (prt)	-

1. 322c-cp is a member of the UPF0057 protein family. It contains potential transmembrane regions (6-23aa; 30-53aa) and could be low temperature or salt-stress inducible.

2. 325c-af shows similarity to IMP4 yeast and related proteins and it might be involved in rRNA processing in *Candida albicans* in a similar way to IMP4.

Claims

1. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 to 9.

2. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 to 3.

3. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule comprises any of the sequences of nucleotides illustrated in Seq ID Nos 1 or 2 and fragments or derivatives of said nucleic acid molecules.

4. A nucleic acid molecule according to any of claims 1 to 3 which is mRNA.

5. A nucleic acid molecule according to any of claims 1 to 3 which is DNA.

6. A nucleic acid molecule according to claim 5 which is cDNA.

7. A nucleic acid molecule capable of hybridising to the molecules according to any of claims 1 to 6 or the sequences illustrated in any of Seq ID Nos 1 to 9 under high stringency conditions.

8. An antisense molecule comprising a nucleic

acid molecule capable of hybridising to the molecules according to any of claims 1 to 6 or the sequences illustrated in any of Seq ID Nos 1 to 9.

9. Cells containing a nucleic acid molecule according to any of claims 1 to 8, wherein said cells are bacterial or eukaryotic.

10. A polypeptide encoded by the nucleic acid molecule according to any of claims 1 to 7 or the sequences illustrated in any of Seq ID Nos 1 to 9.

11. A polypetide having any of amino acid sequences illustrated in any of Seq ID Nos 14 to 20.

12. A recombinant DNA construct comprising a nucleic acid molecule according to claim 5 or 6.

13. A recombinant DNA construct comprising a nucleic acid molecule according to claim 5 or 6 wherein said nucleic acid molecule is inserted in the antisense orientation.

14. A recombinant DNA construct according to claim 12 or 13 wherein said recombinant DNA construct is an expression vector.

15. A construct according to claim 14 which comprises an inducible promoter.

16. A construct according to claim 14 or 15 which comprises a sequence encoding a reporter molecule.

17. Cells containing a recombinant DNA construct according to any of claims 12 to 16, wherein said cells are bacterial or eukaryotic.

18. A nucleic acid molecule according to any of claims 1 to 8 or the nucleotide sequences illustrated in Seq ID Nos 1 to 9 for use as a medicament.

19. Use of a nucleic acid molecule according to any of claims 1 to 8 or the sequences illustrated in Seq ID Nos 1 to 9 in the preparation of a medicament for treating *Candida albicans* associated diseases.

20. A polypeptide according to claim 10 or 11 for use as a medicament.

21. Use of a polypeptide according to claim 10 or 11 in the preparation of a medicament for treating *Candida albicans* associated infections.

22. A pharmaceutical composition comprising a nucleic acid molecule according to any of claims 1 to 8 or a polypeptide according to claim 10 or 11 together with a pharmaceutically acceptable carrier diluent or excipient therefor.

23. A *Candida albicans* cell comprising an induced mutation in the DNA sequence encoding the polypeptide according to claim 10.

24. A method of identifying compounds which selectively modulate expression or functionality of polypeptides or metabolic pathways in which these polypeptides are involved and which are crucial for growth and survival of *Candida albicans*, which method comprises:

(a) contacting a compound to be tested with one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to any of claims 1 to 8 which

mutation results in overexpression or underexpression of said polypeptides in addition to contacting one or more wild type *Candida albicans* cells with said compound,

(b) monitoring the growth and/or activity of said mutated cell compared to said wild type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of said compound on a polypeptide or another polypeptide in the same or a parallel pathway.

25. A compound identifiable according to the method of claim 24.

26. A compound according to claim 25 for use as a medicament.

27. Use of a compound according to claim 25 in the preparation of a medicament for treating *Candida albicans* associated diseases.

28. A pharmaceutical composition comprising a compound according to claim 25 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

29. A method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or organism, which method comprises:

(a) preparing a cDNA or genomic library from said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription

of antisense RNA from the nucleotide sequences in said cDNA or genomic library.

(b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant.

30. A method according to claim 29 wherein said cell or organism is a yeast or filamentous fungus.

31. A method according to claim 29 or 30 wherein said cell or organism is any of *Saccharomyces cerevisiae, Schizosaccharomyces pombe* or *Candida albicans.*

32. An antibody capable of binding to a polypeptide according to claim 10 or 11.

33. An oligonucleotide comprising a fragment of from 10 to 120 contiguous nucleotides of a nucleic acid molecule according to any of claims 1 to 8.

34. An oligonucleotide according to claim 33 comprising a fragment of from 10 to 50 contiguous nucleotides.

FIG. 1.

```
AACAGCTGGT CTTCTGCTAA TACATTCAAC CCTTTCCATA TCTATACTCC
1                                                    50

AACAATATGA TAACTGATGA ACAATTGAAT ACCATTGCAT TGACATTTGG
51                                                  100

TTTTGCTTCA ATAATATTAA TCATAATATA TCATGCCATA TCTACTAATG
101                                                 150

TACATAAATT AGAAGATGAA ACCCCATCAT CTTCATTTAC CAGAACAAAT
151                                                 200

ACTACTGAAA CTACTGTTGC AAGTAAGAAA AAGAAGTAAT AACTGATGGA
201                                                 250

TTTTTCTTCC TACCACCAAT TGAATAATGC TAGACTTGTT GGTGTGCTAC
251                                                 300

AAATATTTCA AAAGAAAATA CGAATACTTT ATAAAATGGT AAGAACGGAA
301                                                 350

GATGGTTTCT CATTTATACA CTAAATACAA ATCACATACA CATACACAAA
351                                                 400

CACAAATACA TACATACACC TATATCCCTT TATTTGAT
401                                      438
```

FIG. 2.

ATGTTAAAAA CACTAACTCA AACTTTACGC TTAACTGGGA AAGCTTTCCC 50
1

AAAGGTCCGT CCGGCCTTGA TCAGAACCTA CGCTGCCTTC GACCGTTCTA 100
51

AACCTCATGT CAACATTGGT ACTATTGGTC ATGTTGATCA TGGTAAAACT 150
101

ACATTGACTG CTGCTATCAC CAAAGTTTTA GCCGAACAAG GTGGTGCCAA 200
151

CTTCTTGGAT TATGGTTCTA TTGATAGAGC TCCAGAAGAA AGAGCTAGAG 250
201

GTATCACTAT TTCCACTGCC CACGTTGAAT ACGAAACCAA GAACAGACAC 300
251

TATGCCCACG TTGATTGTCC AGGACACGCT GATTATATCA AAAATATGAT 350
301

TACTGGTGCC GCTCAAATGG ATGGTGCTAT CATTGTTGTT GCTGCCACTG 400
351

ATGGTCAAAT GCCTCAAACC AGAGAACATT TGTTATTGGC CAGACAAGTT 450
401

GGTGTTCAAG ACTTGGTTGT GTTTGTCAAC AAAGTCGATA CTATTGATGA 500
451

CCCTGAAATG TTGGAATTAG TCGAAATGGA AATGAGAGAA TTGTTATCCA 550
501

CCTACGGTTT TGATGGTGAC AACACTCCAG TTATTATGGG ATCTGCTTTA 600
551

ATGGCTTTGG AAGACAAGAA ACCAGAAATT GGTAAGGAAG CTATCTTGAA 650
601

ATTGTTAGAT GCTGTCGATG AACACATTCC AACTCCATCA AGAGACTTGG 700
651

AACAACCATT TTTGTTACCA GTTGAAGACG TGTTCTCCAT CTCCGGTAGA 750
701

GGAACTGTTG TCACTGGTAG AGTTGAAAGA GGTGTTTTGA AGAAGGGTGA 800
751

AGAAATCGAA ATTGTTGGTG GTTTTGACAA ACCTTACAAG ACTACTGTTA 850
801

CCGGTATTGA AATGTTCAAA AAAGAATTAG ACTCTGCTAT GGCTGGTGAC 900
851

FIG. 2 (CONTINUED).

AACTGTGGTG TTTTGTTAAG AGGTGTTAAA AGAGATGAAA TCAAGAGAGG
901 950

TATGGTTTTG GCCAAACCAG GTACTGCTAC TTCTCACAAG AAGTTCTTGG
951 1000

CTTCCTTGTA TATTTTGACT TCCGAAGAAG GTGGTCGTTC CACTCCATTT
1001 1050

GGTGAAGGTT ACAAGCCTCA ATGCTTCTTC AGAACTAACG ATGTCACTAC
1051 1100

CACATTTTCA TTCCCAGAAG GAGAAGGTGT TGATCATTCT CAAATGATCA
1101 1150

TGCCAGGTGA CAACATTGAA ATGGTTGGTG AATTGATCAA ATCTTGTCCA
1151 1200

TTAGAAGTCA ACCAACGTTT CAACTTGAGA GAAGGTGGTA AAACTGTTGG
1201 1250

TACTGGTTTG ATTACCAGAA TCATCGAATA AACAGAATGT GCACTGTGAA
1251 1300

TAATAAAAAG AAAAGAGGTA TATATAGGTG ACTTTGTATT TTGTATTGAA
1301 1350

CAATAAAATT CTGTAAATAG TAAGGGCCTC
1351 1380

FIG. 3.

GAATTCGCCCTTAAGCACTCGTTTCAACTATACATTCAGTAACAACACCCTTAATTTACCAAACTACA
TTAATGGAAGTA
ACACAACGGACGCAGAGTCAGACACAACCAACACAACAGTCACCGACAACTCAGACGCAAACCCAAAG
CAAAGAGGACCA
GAATAGGATTTGTCAATTGATTTGCTCCACGGGTCAGTTTGGCAATTATGATTTGAATATCAACGATA
AAACTATCGTAC
AAGGTAAAATGACGTGGTATTTTGGAAGAGACCCCAACTCAGATTTGCAAGTGGCGTCGTCGTCGAGA
ATTTCAAACAAG
CATTTTCAAATCTGGCTCAACTTCAATGATAAATCACTATGGATAAAGGACACTTCAACTAACGGGAC
ACACCTTAACAA
CAGTCGATTGGTGAAAGGATCAAACTACCTTCTTAATCAGGGTGATGAAATAGCAGTAGGGGTTGGTA
GAGACGAGGACG
TTGTGAGGTTTGTCGTTGTCTTTGGTGACAAATACAACCCGGCAAAGCTACCTGATTCGACCAACACA
ATTAAAGATGAA
GGAATATACAAAGACTTTATTGTGAAAAATGAAACGATAGGCCAAGGAGCATTTGCCACTGTGAAAAA
GGCGATTGAACG
ATCTACGGGCGAGTCGTACGCGGTGAAGATTATAAATCGAAGAAAAGCATTAAATACCGGTGGTGGAA
GTGCCATGGCAG
GAGTGGACCGTGAATTGTCCATATTAGAGCGGCTCAACCACCCAAATATAGTTGCTCTAAAAGCTTTT
TATGAAGATATG
GACAATTACTATATTGTGATGGAATTGGTGCCGGGCGGTGATTTGATGGACTTTGTGGCTGCAAACGG
TGCAATAGGAGA
AGACGCAACACAAGTGATCACGAAACAGATTCTAGAAGGAATTGCCTATGTTCATAATTTAGGAATCT
CCCATCGTGATT
TGAAGCCAGATAATATTTTGATTATGCAAGATGACCCAATACTTGTTAAAATCACCGACTTTGGATTG
GCAAAATTCAGT
GACAATCTGACGTTTATGAAAACTTTTTGTGGTACATTGGCGTATGTTGCTCCCGAAGTTATCACCGG
TAAGTATGGATC
ATCGCAGATGGAACTGCAACAAAAGGACAACTACTCTTCCTTGGTTGACATTTGGTCTTTGGGATGTT
TGGTTTATGTAC
TTTTAACTTCTCATTTACCATTCAACGGGAAAAACCAGCAACAAATGTTTGCCAAGATCAAAAGGGGC
GAATTTCATGAG
GCTCCATTAAATTCATACGACATTTCTGAAGACGGAAGAGATTTCTTGCAGTGCTGCCTACAGGTTAA
TCCTAAACTAAG
GATGACGGCTGCTGAAGCTTTGAAACATAAATGGTTGCAAGACTTGTATGAAGAGGATTCTGTCAAAT
CATTGAGTTTAT
CGCAATCACAGTCGCAACAATCTCGAAAGATAGATAATGGTATCCATATCGAATCATTGAGCAAAATT
GATGAAGACGTT
ATGCTTCGTCCATTGGATAGCGAAAGAAATAGGAAATCAAGTAAACAGCAAGATTTCAAGGTACCCAA
GCGTGTGATTCC
GTTATCTCAACATCCTGCAACACCGTTACCAATGTCACAACCGAAAAAGAGGCCGTATCAAATAGACC
CTAGAACAAACA
AAAAAGTCGATTTGGAAGAACCTCTGACAAGCAAGAAAGTCAAGCTAAGTGATTCCGTTGTTGCGGAA
GACTACTTGAAG
TTGGGGCCACTTGCAAATTCGTTATTCCAAGAAACAATAAATATTTCAAAGTCCCCGTTTTCTTTCGG
AAGAAATGACAC
TTGTGATTGCGAGATAGACGACGACAGACTATCCAAACTTCATTGTGTCATTACCAAAGAAAACGACT
CTATATGGTTAT
TGGATAAGAGTACTAACTCGTGCTTGGTCAACAATACTAGTGTTGGAAAAGGCAACAAAGTTTTGCTT
AGAGGAGGGGAG
ATATTACATCTCTTCTTTGACCCATTGTCACTGCAACATATAGGTTTCAAAGTAGTCCTTGTTGATCA
ACTGTCTGGTGA
ACATAAGAGTCAAGTGGAGGTTTTGAAACAAACCTCAGAAGAAATGAATATTATTCCACTTATTTCTG
GTTTAAGTAGTA
TAAGTTCATAGATTTAGCATATATACAAGCATTTCCTATAGAAACAAAGGTTCATTAATTTAGTTATT
TACCTCCATGCA
ATTACATTTACTTCTTCTTCCAAGGGCGAATTCTGCAGATATC

FIG. 4.

ATGGGTAGTATGTGAAGATACAATATTGAAAGTGTTTACTAGAATATCTAAGATGTTTGAGCCCATGG
AC
ATTTTTGGATTTGATAATTAAAAAAAGTAGCAATAGATTATTGCGTTGGAGAAAGAATCACCATAGTT
GC
AAGATTTGATAGATGTTAAAATGTTCACGCAGGCGAAAGATGTAACATCTCTTAAAGTAAGAAGAATA
TG
GACATGAATAAAAATAGATAGCACTATTTTGGAACTTGTTGAAGATATTAAAATAGAATGGGATTTCA
AC
ATAGATATTCAAAGTAACGAAACCTCACAATCAAATAAAAACAACAGTAATACTAACAATTCAATTTT
TA
TTTTTATAGAGGGTACTCCATCTTTAGGTAAACGTCACAACAAATCTCACACCTTATGTAACAGATGT
GG
CCGTCGTTCATTCCACGTCCAAAAGAAGACCTGTTCTTCTTGTGGTTACCCAGCTGCTAAAATGAGAT
CT
CACAACTGGGCCTTAAAAGCCAAAAGAAGAAGAACTACTGGTACCGGTAGAATGGCTTACTTGAAACA
CG
TTACCAGAAGATTCAAGAACGGTTTCCAAACTGGTGTTGCTAAAGCTCAAACCCCTTCCGCTTAAACT
AA
TTACTGAAGTTATTGGTCATGCATTAGTCATTATTCATTAAAGTCATGTTAAGCATAGCAAAGGAAGA
AT
TGGTTAGATTCTTGTTTAAAATGTAATGACTATTTAATATCTGTTTAAATAAGAGGTTTAGTCTTTAT
TT
TTTTACGTATACACCAAAAAAAAAAGAAACAAATAAAATCTGTATATTAATGTTGG

FIG. 5.

ATGGGTACTA GTACAAGTGA AGCATTGAAG AACATCAAAA ACAAACAGCG 50
1

AAGACAGAAA GTTTTTGCAG AAATAAAACA TGAAAAGAAT AAACAACGTC 100
51

ATAAGCAAAG AGCCGAAAGA GCTAAGGAAG AAAGAGAAAA CCCCGAATTA 150
101

AGAGAGGAAA GAATAGCAGC TAATATCCCA GATACTATAG ATAGCAAACG 200
151

TATTTATGAT GAGACTATAG CTGCTGAAGT TGAAGGAGAT GACGAGTTTC 250
201

AGTCATATTT CACTAACTTG TTGGAAGAAC CAAAGATTTT GTTGACAACA 300
251

AGTGCCAATG CTAAAAAACC GGCCTATGAA TTTGCAGACA TGATCATGGA 350
301

CTTTTTACCG AATGTGACAT TTATCAAAAG GAAGAAGGAA TATACAATGC 400
351

AAGATATGGC CAAATATTGC TCGAATAGAG ACTTCACTGC ATTGCTTGTC 450
401

ATCAACGAAG ACAAGAAGAA GGTCAATGGT ATAACGCTCA TCAATTTACC 500
451

TGAAGGGCCA ACATTTTATT TTTCGATTAC ATCAATAGTT GATGGGAAAA 550
501

GAATTAAGGG ACACGGGAAA GCTGGTGATT ATTTACCTGA GATTGTATTG 600
551

AATAATTTCA ATTCAAGATT GGGTAAAACT GTGGGAAGAC TATTTCAAAG 650
601

TATTTTCCCT CATAAACCTG AACTTCAAGG AAGACAAGTG ATTACTTTGC 700
651

ACAATCAACG TGATTATATT TTTTTCAGAA GACATAGATA TATTTTCAGA 750
701

AATGAGGAAA AGGTTGGATT GCAGGAATTG GGTCCGCAGT TTACATTAAA 800
751

GCTAAGAAGA ATGCAAAAGG GAGTACGTGG TGATGTTGTT TGGGAACACA 850
801

GACCAGATAT GGAAAGAGAT AAGAAGAAGT TTTATTTATA AGCGGGTGTA 900
851

FIG. 5 (CONTINUED).

```
TAAAGGTAGT AGTAGTGCGT TTATAAGTAT GTGTGTGTGT TTATGCATAG
901                                                  950

ATGTGTAAAG AGTAATACAG CTAATTCG
951                          978
```

FIG. 6.

AACTAATTTG TTTAAACATC AATACCAAGA AGTTTTTACA ATTCAATCCC
1 50

ACATACACCA TTAATTATGA ATTCTGAAAA GATTATTGAA GTTATCATTG
51 100

CTATTTTCTT ACCACCAGTA GCTGTGTTTA TGAAATGTGG TGCCACTACC
101 150

CCATTATGGA TTAACTTGGT ATTATGTATC TTTATTTGGT TCCCTGCTAT
151 200

CTTACATGCC TTATACGTTG TGTTGAAAGA TTAAACAAAC ACCAGAGATT
201 250

TACTGCTTGA TGAATTGATT ACTCCAAAGA GTTGTGACTA GTTCCCAGTG
251 300

TGTTTTTTTT GCCTTCCAAC TTTCTTTTAC ATTTTTCCAT TACTACCACT
301 350

GTCTTCCCCC CTATTTTGCA GAGTTTTCAA AATTTATCCA AAACATGTTA
351 400

GTCATTAAAC CATATTATTA TAATTATTCT TTTTTGTATT TTTTTCCCTT
401 450

AAAACACGTT AATTTATTAA TCGTTTCGTT GTTTGGTATT TTATTTTTTT
451 500

GTATTTATCA ATTGGAATAT ATATCTATAC ATGAATTTAT TATCCATTGT
501 550

ACCAATTGTT AAAACATTTT GTTAGTTTTT TGTTACTAGT ATAAAANNAT
551 600

AATAAAAGTT TANTTCAAC
601 619

FIG. 7.

ATGACATTAG GGTTCGATAA ATTCATAAGC AAGGTCAGCA CTCATAGACG
1 50

TCAATCTGAA CCATCAATCT TGGAAATCGC AGCCACCAAT TCTCAAAATA
51 100

AATCGAGAAG GCTAAGTATG GATAATGGTC ATTGTTATGT TCGTGAATCA
101 150

ACTAATAATC ATCATCATTT AAATACCGTC GTTGATAATT TACGACAGCG
151 200

TGCGGGATCG TTTTCATTTA TTTCACATCA CCATAATCAC CATCAGAATA
201 250

GTCACGATAA TTATACTGTC GATCCCCTTA CATCAAACGG AGCACGAATT
251 300

TCCCGATCAC GTTCACGTTC CAAATCAGTT GGGCACGGAG AAGCAATATC
301 350

ACCAGCGTAT TTTTCCAAGA ATAAAACCAA AGATTTAGTG AAACAGGAAA
351 400

CAGCACATAT CATTCTGAAG AAATTACTCA ACATGTTACA AGATTTGGAT
401 450

TTACAAAACC CTATTGCATT GAAAACAATA TCACAAGGTT CAGAATCAAA
451 500

GTTTTGTAAA ATCTACGTGT CTAACACTAA TAATTGTATT TACTTACCAG
501 550

CAGCAAGTTC AACAAGTTTC ACTTATGAAG ATGATGAAAA TGGCGGCGTT
551 600

ATAATTGCTG AAGATAGAAA TGATGAAATG CCAACAGCAG TTAATAACAA
601 650

TACTTTGTCA ATGGATAGTA TAAATCATTC AGAGACTGAT TTCCTGGATT
651 700

CTCCACCACC TCCAGATTTA TTTTCTAAAA TGAAATCATT CCATTCACCA
701 750

AATTACTTGA CTTCAAAAAT CGATTCTGAA TGTCCAATTC CACATACATT
751 800

TGCTGTGATT GTTGAATTAA CCAAGGACTC TTTGATTATT AAAGATCTTC
801 850

ATTTCCAATT TCAGTCATTA ACTACCATTT TATGGCCAAC TGGGGATGCA
851 900

FIG. 7 (CONTINUED 1).

TATAATCGGA CTCATGCCAA GGAGAAATTT ACCATTGGGA ATATGGAATG
901 950

GCGTACATCT TTAAGCGACG CCGACTATTA TATCAATAGT TCTAATTCCA
951 1000

ACGATGTTAA GCTGAAAAAC TTGGGTCCTG AAGATCTTAT TAATCGAACT
1001 1050

AGAGAATACA AATTAATCGA TATTGAAGAA CCAAACAATT CATCAAACAG
1051 1100

TTTACTGGAT GATGACATGG ATATTAATAA TATTACGTCG CCATTATCAA
1101 1150

CGTCACCAAC ATCAAGTTCA ACTTCAACAA ATTCAACCTC CAACTCATTG
1151 1200

GGTTCAGATT CATATAAAGC TGGTCTTTAT GTATTTTTAT TACCAATCTT
1201 1250

ATTGCCAGAA CATATTCCTG CTTCCATTGT TTCTATTAAT GGTTCATTGG
1251 1300

CTCATACATT ACTGGTTGAA TGCAATAAAT ATACTGATAA GTTGAATCGG
1301 1350

AAATCAAAAG TATCAGCATC GTACAATTTA CCTATGGTCC GTACTCCACC
1351 1400

AAACATTGGT AATTCCATTG CTGATAAGCC AATTTATGTT AATAGGATTT
1401 1450

GGAATGATGC CGTACATTAT ATTATAACTT TCCCCCGCAA ATATGTTACT
1451 1500

TTGGGTTGTG AACACATGAT AAATGTGAAA TTACTGCCCA TGGTGAAAGA
1501 1550

TGTGGTTATC AAGCGTATTA AATTTAAIGT ATTGGAGAGA ATAACTTATG
1551 1600

TTTCCAAAAA TTTATCACGA GAATATGATT ATGATAGTGA AGACCCCTAT
1601 1650

TGTATTCATC CAGTTTCTAA AGAAAATAAA GTACGTGAAC GTGTTGTGTC
1651 1700

GTTATATGAA TTGAAAACGA AGGCAAAACA ATCTTCTGGT GGACATCTTG
1701 1750

AAGCTTATAA ACAAGAAGTT ATGAAATGTC CGGAAAATAA CCTTTTATTT
1751 1800

FIG. 7(CONTINUED 2)

TCTTGTTATG AGGTTGAAAA TGATAATAAT AACGGCAACG GCAACGGCAA
1801 1850

CGGCAACGGA AACAAGAACG TTAAACAAAA GAATAAAGAT CAACCAATGA
1851 1900

TTGCTACACC TTTAGATATC AATGTTTCTT TACCATTTTT AACTACTATG
1901 1950

TCTGATAGTT TAATTATGAC ATCAGCCATA GAAGAAGAAG GTTCAGATCT
1951 2000

GCCTCATACA TCAAGAAGAG GGTCGGCAGT GAGTATGACT GATAATAATA
2001 2050

CTACCCCAAG TAACAATAAC CCTTTATCTC CATTTTTGGG AGCAGTGGAA
2051 2100

ACTAATGGTG CTAGTATAAA TGAAATTGGT GATCATACAT TATTCCCTGA
2101 2150

TTCTAATTTT CGACATATTG AAATTAAACA TCGATTACAA GTTACATTTA
2151 2200

GGATTTCTAA ACCGGATCTG GATAATAAAA TGCATCATTA TGAAGTGGTT
2201 2250

ATTGATACCC CCATCGTTTT ACTTAGTTCA AAATGTCAAG AAGATTCTCC
2251 2300

TCCTCCTTAT AGTTCTGTA
2301 2319

FIG. 8.

AACGTTCGTG CAAAAGGCTA TACTGGTGAT ATCCACGCAG ATGAAGAGCA
1 50

AGTTTAATCA ACTCTTTGTC AATTAATGCT GTACTTGTTT TCATTTTATT
51 100

TGCTGGCATT TAAAGAATAC CCATAGTTCA GAAAATAAAA TTGAAAAATT
101 150

TAAAAAAAAA CGCAATATCA TTCATTTTTT TTGTTTTTTT GACAATAATA
151 200

TTAATATGTA GTTACCAATG TTTTTAGATT TTATATGTTT TGAAAAAATA
201 250

GTTTG
251

FIG. 9.

AACCTTACAA TCATTATACC AACTATCAAA ATCATAAGAC TCTTNAACTT
1 50

CTGTTTTTGA TAGTTGGTAT AATGATTTAT GTATTATCTT AATTCATTAT
51 100

TATTAGTTTC GGTCACAAA
101 119

FIG. 10.

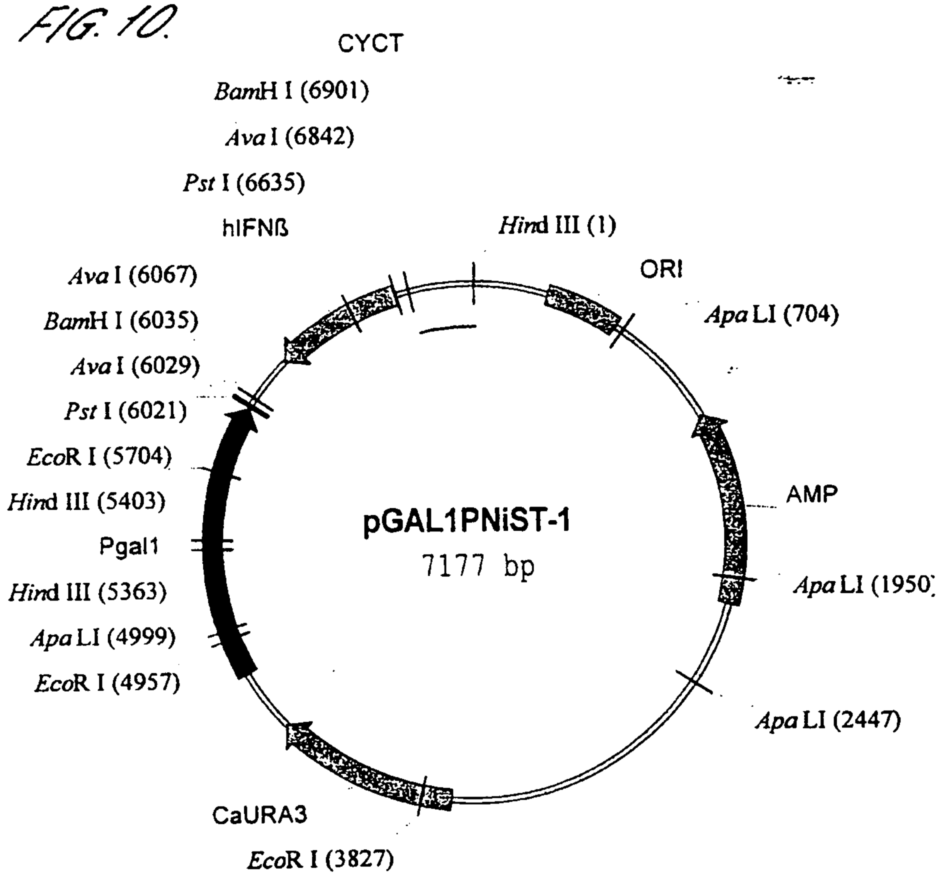

CYCT
BamH I (6901)
Ava I (6842)
Pst I (6635)
hIFNß
Hind III (1)
ORI
Ava I (6067)
BamH I (6035)
Ava I (6029)
Pst I (6021)
EcoR I (5704)
Hind III (5403)
Pgal1
Hind III (5363)
ApaLI (4999)
EcoR I (4957)
ApaLI (704)
AMP
ApaLI (1950)
ApaLI (2447)
pGAL1PNiST-1
7177 bp
CaURA3
EcoR I (3827)

FIG. 11.

1 AGCTTGAGTA TTCTATAGTG TCACCTAAAT AGCTTGGCGT AATCATGGTC
51 ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA
101 TACGAGCCGG AAGCATAAAG TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC
151 TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA
201 CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG
251 GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC
301 TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT
351 ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA
401 AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT
451 TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG
501 TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC
551 CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA
601 TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC
651 ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT
701 GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC
751 TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC
801 AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG
851 AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT
901 GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG
951 CTCTTGATCC GGCAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT
1001 GCAAGCAGCA GATTACGCGC AGAAAAAAAG GATCTCAAGA AGATCCTTTG
1051 ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT CACGTTAAGG
1101 GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG ATCCTTTTAA
1151 ATTAAAAATG AAGTTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG
1201 TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG
1251 TCTATTTCGT TCATCCATAG TTGCCTGACT CCCCGTCGTG TAGATAACTA
1301 CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA

FIG. 11 (CONTINUED 1).

1351 GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC AGCCAGCCGG
1401 AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT
1451 CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT
1501 TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC
1551 GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA
1601 CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG
1651 ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC
1701 AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG
1751 TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA
1801 CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG
1851 CAGAACTTTA AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC
1901 TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT
1951 GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG
2001 AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC
2051 GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT
2101 TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA
2151 AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCTG
2201 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT
2251 ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG GTGAAAACCT
2301 CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG TAAGCGGATG
2351 CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT
2401 CGGGGCTGGC TTAACTATGC GGCATCAGAG CAGATTGTAC TGAGAGTGCA
2451 CCATATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA AAATACCGCA
2501 TCAGGCGAAA TTGTAAACGT TAATATTTTG TTAAAATTCG CGTTAAATAT
2551 TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC
2601 CTTATAAATC AAAAGAATAG ACCGAGATAG GGTTGAGTGT TGTTCCAGTT
2651 TGGAACAAGA GTCCACTATT AAAGAACGTG GACTCCAACG TCAAAGGGCG

FIG. 11 (CONTINUED 2).

2701 AAAAACCGTC TATCAGGGCG ATGGCCCACT ACGTGAACCA TCACCCAAAT
2751 CAAGTTTTTT GCGGTCGAGG TGCCGTAAAG CTCTAAATCG GAACCCTAAA
2801 GGGAGCCCCC GATTTAGAGC TTGACGGGGA AAGCCGGCGA ACGTGGCGAG
2851 AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG CTGGCAAGTG
2901 TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG
2951 CTACAGGGCG CGTCCATTCG CCATTCAGGC TGCGCAACTG TTGGGAAGGG
3001 CGATCGGTGC GGGCCTCTTC GCTATTACGC CAGCTGGCGA AAGGGGGATG
3051 TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC CAGTCACGAC
3101 GTTGTAAAAC GACGGCCAGT GAATTGTAAT ACGACTCACT ATAGGGCGAA
3151 TTGGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG TGGCGCGGTA
3201 TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA
3251 TTCTCAGAAT GACTTGGTTG AGTACTAATA GGAATTGATT TGGATGGTAT
3301 AAACGGAAAC AAAAAAAAGA GCTGGTACTA CTTTCTTTAA AATTATTTTA
3351 TTATTTGATT TTATTTAATA GTATATATTA TATTTTGAAC GTAGATTATT
3401 TTGTTGAAAG TTGCTGTAGT GCCATTGATT CGTAACACTA ATTCTGTATT
3451 AGTCATTCCT CTTGTTTGAT AGTATCCAAA AAAACGGCTA TTTTTTTGCA
3501 ATCTTATTTC CTGCATATTA TACAGATAAC ATAATGAAAG AAAAAATCTT
3551 TTTTTTTGTT CTTCAATGAT GATTTCAACC ATTCTTTTAA ACATTGATCA
3601 ATTCCTGAGC AACAACCCCA TACACACTGG TTTATATACC GCCCCTTTTA
3651 CAGTTGAAGA AAGAAATAGA AATAGAAATA GCAAACAAAA GATATGACAG
3701 TCAACACTAA GACCTATAGT GAGAGAGCAG AAACTCATGC CTCACCAGTA
3751 GCACAGCGAT TATTTCGATT AATGGAACTG AAGAAAACCA ATTTATGTGC
3801 ATCAATTGAC GTTGATACCA CTAAGGAATT CCTTGAATTA ATTGATAAAT
3851 TAGGTCCTTA TGTATGCTTA ATCAAGACTC ATATTGATAT AATCAATGAT
3901 TTTTCCTATG AATCCACTAT TGAACCATTA TTAGAACTTT CACGTAAACA
3951 TCAATTTATG ATTTTTGAAG ATAGAAAATT TGCTGATATT GGTAATACCG
4001 TAAAGAAACA ATATATTGGT GGAGTTTATA AAATTAGTAG TTGGGCAGAT

FIG. 11 (CONTINUED 3).

4051 ATTACCAATG CTCATGGTGT CACTGGGAAT GGAGTGGTTG AAGGATTAAA
4101 ACAGGGAGCT AAAGAAACCA CCACCAACCA AGAGCCAAGA GGGTTATTGA
4151 TGTTAGCTGA ATTATCATCA GTGGGATCAT TAGCATATGG AGAATATTCT
4201 CAAAAAACTG TTGAAATTGC TAAATCCGAT AAGGAATTTG TTATTGGATT
4251 TATTGCCCAA CGTGATATGG GTGGCCAAGA AGAAGGATTT GATTGGCTTA
4301 TTATGACACC TGGAGTTGGA TTAGATGATA AAGGTGATGG ATTAGGACAA
4351 CAATATAGAA CTGTTGATGA AGTTGTTAGC ACTGGAACTG ATATTATCAT
4401 TGTTGGTAGA GGATTGTTTG GTAAAGGAAG AGATCCAGAT ATTGAAGGTA
4451 AAAGGTATAG AAATGCTGGT TGGAATGCTT ATTTGAAAAA GACTGGCCAA
4501 TTATAAATGT GAAGGGGGAG ATTTTCACTT TATTAGATTT GTATATATGT
4551 AGAATAAATA AATAAATAAG TTAAATAAAT AATTAAATAA GGGTGGTAAT
4601 TATTACTATT TACAATCAAA GGTGGTCCTT CTAGCTGTAA TCCGGGCAGC
4651 GCAACGGAAC ATTCATCAGT GTAAAAATGG AATCAATAAA GCCCTGCGCA
4701 GCGCGCAGGG TCAGCCTGAA TACGCGTTTA ATGACCAGCA CAGTCGTGAT
4751 GGCAAGGTCA GAATAGCCCA AGTCGGCCGA GGGGCCTGTA CAGTGAGGGA
4801 AGATCTGATA TTGACGAAGA GGAACCAATG TAACGTTACA CTGAAGAAAA
4851 CACATAATAA ACGGGAAGAA ACGGTGTAAA AGTGTGAAAA TAATTTTTGA
4901 ATATCATTTC CCTTGGTTTA ATTCCAAACG AAACGTGTAT TTTTTTAGAG
4951 AATGGGAATT CTTATTGGAT GTCTAGATTG TTTGTTTACT CCAGACTGTG
5001 CACAAAAACG TTTGGATGGA TGATCAGAAG ATATTTTTAG GCTTAGCTCT
5051 AAATATAAGA AATGATGCTT GAAAATCCAG ACAGAAATTG AGTTTCAAAA
5101 ATTGGTAATG TGAGGTATTA GTCAACTAAC CAAATAACAA TGCAAACCGG
5151 TTGATACATT TCATTTTGAA AATAATGAAA CTGGAATTGG ATGACCAGCA
5201 CACAAACACA TAAAGTAATT ATGGGAATTA GAAGCGAACA TAGAGGAATA
5251 CTTTGCCACG AACAGAATAC AAGTGGGAAC ACTTTTTTCT CCATTGTTTT
5301 AGTTCTGTTT TTTTGTCAAA CTGGTTTTGT GCTATGTGTA AAAAAATATT
5351 GCCAAGAAAA AAAGCTTGTT TTGTGCCCAG TGTCCGAAAA AAATTTTGGG

FIG. 11 (CONTINUED 4).

5401 GAAGCTTCGG ATTAATTTAT TTTTTTATTC CATCGGGGAA AGTGGGGGGG
5451 AAAAAAAATT TAAGCAGTTC ATAAAACCTT CCAAAAAATA TATGGACAGA
5501 GATGATTGTA TTTTCCCGAC ACCAAAATCA TAATTAACTA TGAGAAAATT
5551 GAATGTAACG TTACAATTTA TTTTTATTTG AAGCTGAAAA GCGATTTATG
5601 ATTTTTCCGA AATGAAAATT TTTTTTAGGT TTATTTTTTT TGTCGGGCAA
5651 AGAAAAACTG AACAAGGATT ATTAAAATTT TTGGTGTTTG TTTGTGTCTG
5701 GAGAATTCAT TCCTCTCTCA TCTTCACACA ATGTTTAGAC ATCTGACACG
5751 ATTCAAAATA GTTCGGTTTC CGGGGTTGGT GTTTAGTTTT CGTTTTTCGT
5801 TTTTTTTGGA AAGAATGTTT TAGCTCATTG GTTTTCTTTC TTCATTCAAT
5851 AGTTTTGAAA GAATTTGCCC ACTTGTTATT ACAATCATAT AAAATTAAAC
5901 TTTGATATAA AATAGAGTTT GAAAGTTTCC CAGATCCTTT TTGATTTCTT
5951 TGTAATTTTT TTTTCTCCCA CATATACACA CATACAAACC GATTTTTATA
6001 AGAAAGAGTT ATACCCTGCA GCTCGACCTC GAGGGATCCG GGCCCTCTAG
6051 ATGCGGCCGC TAGGCCTCGA GGGACTTTTG CACCAAAAAT AATTTATTTT
6101 CCAAAATAAA ATTTAAATAA ATAAAAATAA CTCATAATTT AATAAAAATT
6151 TCAAAATCTT CTAGTGTCCT TTCATATGCA GTACATTAGC CATCAGTCAC
6201 TTAAACAGCA TCTGCTGGTT GAAGAATGCT TGAAGCAATT GTCCAGTCCC
6251 AGAGGCACAG GCTAGGAGAT CTTCAGTTTC GGAGGTAACC TGTAAGTCTG
6301 TTAATGAAGT AAAAGTTCCT TAGGATTTCC ACTCTGACTA TGGTCCAGGC
6351 ACAGTGACTG TACTCCTTGG CCTTCAGGTA ATGCAGAATC CTCCCATAAT
6401 ATCTTTTCAG GTGCAGACTG CTCATGAGTT TTCCCCTGGT GAAATCTTCT
6451 TTCTCCAGTT TTTCTTCCAG GACTGTCTTC AGATGGTTTA TCTGATGATA
6501 GACATTAGCC AGGAGGTTCT CAACAATAGT CTCATTCCAG CCAGTGCTAG
6551 ATGAATCTTG TCTGAAAATA GCAAAGATGT TCTGGAGCAT CTCATAGATG
6601 GTCAATGCGG CGTCCTCCTT CTGGAACTGC TGCAGCTGCT TAATCTCCTC
6651 AGGGATGTCA AAGTTCATCC TGTCCTTGAG GCAGTATTCA AGCCTCCCAT
6701 TCAATTGCCA CAGGAGCTTC TGACACTGAA AATTGCTGCT TCTTTGTAGG

FIG. 11 (CONTINUED 5).

```
6751  AATCCAAGCA AGTTGTAGCT CATGGAAAGA GCTGTAGTGG AGAAGCACAA
6801  CAGGAGAGCA ATTTGGAGGA GACACTTGTT GGTCATGTTC CTCGAGGCCT
6851  TTTTGGCCAG CTGGCGCCTG CTGCGCGACG GCGAGCTGCT CACCACCCAG
6901  GATCCGTCCC CCTTTTCCTT TGTCGATATC ATGTAATTAG TTATGTCACG
6951  CTTACATTCA CGCCCTCCCC CCACATCCGC TCTAACCGAA AAGGAAGGAG
7001  TTAGACAACC TGAAGTCTAG GTCCCTATTT ATTTTTTTAT AGTTATGTTA
7051  GTATTAAGAA CGTTATTTAT ATTTCAAATT TTTCTTTTTT TTCTGTACAG
7101  ACGCGTGTAC GCATGTAACA TTATACTGAA AACCTTGCTT GAGAAGGTTT
7151  TGGGACGCTC GAAGGCTTTA ATTTGCA
```

FIG. 12.

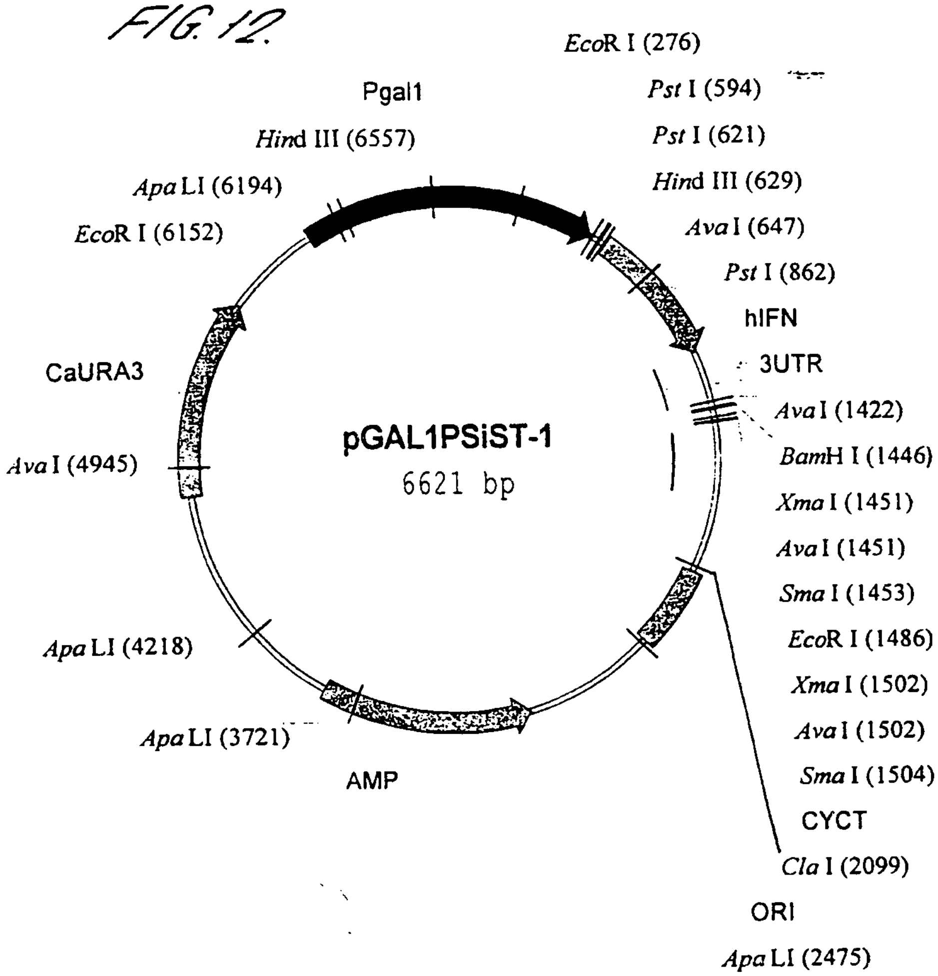

EcoR I (276)
Pst I (594)
Pst I (621)
Hind III (629)
Ava I (647)
Pst I (862)
hIFN
3UTR
Ava I (1422)
BamH I (1446)
Xma I (1451)
Ava I (1451)
Sma I (1453)
EcoR I (1486)
Xma I (1502)
Ava I (1502)
Sma I (1504)
CYCT
Cla I (2099)
ORI
Apa LI (2475)
Pgal1
Hind III (6557)
Apa LI (6194)
EcoR I (6152)
CaURA3
Ava I (4945)
pGAL1PSiST-1
6621 bp
Apa LI (4218)
Apa LI (3721)
AMP

FIG. 13.

1 TTCCATCGGG GAAAGTGGGG GGGAAAAAAT TTTAAGCAGT TCACAAAACC
51 TTCCAAAAAA TATATGGACA AAGATGATTG TATTTTCCCG ACACCAAAAT
101 CATAATTAAT TATGAGAAAG TTAAATGTAA CGTTACAATT TATGTTTATT
151 TGAAGGTGAA AAGCGATTTA TGATTTTTCC GAAATGAAAA TTTTTTTTAG
201 GTTTATTTTT TTTGTCGGGC AAAGAAAAAC TGAACAAGGA TTATTAAAAT
251 TTTTGGTGTT TGTTTGTGTC TGGAGAATTC ATTCCTCTCT CATCTTCACA
301 CAATGTTTAG ACATCTGACA CGATTCATGA TAGTTCGGTT TCCGGGGTTG
351 GTGTTTAGTT TTCGTTTTTC TTTTTTTTTG GAAAGAATGT TTTAGCTCAT
401 TGGTTTTCTT TCTTCATTCA ATAGTTTTGA AAGAATTTGC CCACTTGTTA
451 TTACAATCAT ATAAAATTAA ACTTTGATAT AAAATAGAGT TTGAAAGTTT
501 CCCAGATCCT TTTTGATTTC TTTGTAAATT TTTTTTTCTC CCACATATAC
551 ACACATACAA ACCGATTTTT ATAAGAAAGA GTTATACCCT GCAGCTCGAC
601 CTCGACTGTT TAAACCTGCA GGCATGCAAG CTTGGCCAAA AAGGCCTCGA
651 GGAACATGAC CAACAAGTGT CTCCTCCAAA TTGCTCTCCT GTTGTGCTTC
701 TCCACTACAG CTCTTTCCAT GAGCTACAAC TTGCTTGGAT TCCTACAAAG
751 AAGCAGCAAT TTTCAGTGTC AGAAGCTCCT GTGGCAATTG AATGGGAGGC
801 TTGAATACTG CCTCAAGGAC AGGATGAACT TTGACATCCC TGAGGAGATT
851 AAGCAGCTGC AGCAGTTCCA GAAGGAGGAC GCCGCATTGA CCATCTATGA
901 GATGCTCCAG AACATCTTTG CTATTTTCAG ACAAGATTCA TCTAGCACTG
951 GCTGGAATGA GACTATTGTT GAGAACCTCC TGGCTAATGT CTATCATCAG
1001 ATAAACCATC TGAAGACAGT CCTGGAAGAA AAACTGGAGA AAGAAGATTT
1051 CACCAGGGGA AAACTCATGA GCAGTCTGCA CCTGAAAAGA TATTATGGGA
1101 GGATTCTGCA TTACCTGAAG GCCAAGGAGT ACAGTCACTG TGCCTGGACC
1151 ATAGTCAGAG TGGAAATCCT AAGGAACTTT TACTTCATTA ACAGACTTAC
1201 AGGTTACCTC CGAAACTGAA GATCTCCTAG CCTGTGCCTC TGGGACTGGA
1251 CAATTGCTTC AAGCATTCTT CAACCAGCAG ATGCTGTTTA AGTGACTGAT
1301 GGCTAATGTA CTGCATATGA AAGGACACTA GAAGATTTTG AAATTTTTAT

FIG. 13 (CONTINUED 1).

1351 TAAATTATGA GTTATTTTTA TTTATTTAAA TTTTATTTTG GAAAATAAAT
1401 TATTTTTGGT GCAAAAGTCC CTCGAGGCCT AGCGGCCGCC TAGAGGATCC
1451 CCGGGCGCTA GGCGGCCGCT AGGCCTTTTT GGCCGAATTC GAGCTCGGTA
1501 CCCGGGGAGA TCCGTCCCCC TTTTCCTTTG TCGATATCAT GTAATTAGTT
1551 ATGTCACGCT TACATTCACG CCCTCCCCCC ACATCCGCTC TAACCGAAAA
1601 GGAAGGAGTT AGACAACCTG AAGTCTAGGT CCCTATTTAT TTTTTTATAG
1651 TTATGTTAGT ATTAAGAACG TTATTTATAT TTCAAATTTT TCTTTTTTTT
1701 CTGTACAGAC GCGTGTACGC ATGTAACATT ATACTGAAAA CCTTGCTTGA
1751 GAAGGTTTTG GGACGCTCGA AGGCTTTAAT TTGCAAGCTA GCTTGGCGTA
1801 ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG CTCACAATTC
1851 CACACAACAT ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA
1901 TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA
1951 GTCGGGAAAC CTGTCGTGCC AGAGATCTCT GCATTAATGA ATCGGCCAAC
2001 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC
2051 ACTGACTCGC TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGATCG
2101 ATCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG
2151 CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA
2201 AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA
2251 TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT
2301 AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT
2351 CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG
2401 CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
2451 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC
2501 CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
2551 CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
2601 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
2651 TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC

FIG. 13 (CONTINUED 2).

2701 CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
2751 GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA
2801 GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG
2851 GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA
2901 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
2951 AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA
3001 GGCACCTATC TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC
3051 TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC
3101 AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC
3151 AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA
3201 CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA
3251 AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG
3301 CATCGTGGTG TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT
3351 CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG
3401 GTTAGCTCCT TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT
3451 GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC
3501 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC
3551 TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG
3601 GGATAATACC GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA
3651 AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC
3701 AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC
3751 TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA
3801 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT
3851 TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA
3901 CATATTTGAA TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT
3951 TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA
4001 TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC TCGCGCGTTT

FIG. 13 (CONTINUED 3).

```
4051  CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
4101  CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG
4151  TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA
4201  GCAGATTGTA CTGAGAGTGC ACCATATCGA CGCTCTCCCT TATGCGACTC
4251  CTGCATTAGG AAGCAGCCCA GTAGTAGGTT GAGGCCGTTG AGCACCGCCG
4301  CCGCAAGGAA TGGTGCATGC AAGGAGATGG CGCCCAACAG TCCCCCGGCC
4351  ACGGGGCCTG CCACCATACC CACGCCGAAA CAAGCACTAA TAGGAATTGA
4401  TTTGGATGGT ATAAACGGAA ACAAAAAAAA GAGCTGGTAC TACTTTCTTT
4451  AAAATTATTT TATTATTTGA TTTTATTTAA TAGTATATAT TATATTTTGA
4501  ACGTAGATTA TTTTGTTGAA AGTTGCTGTA GTGCCATTGA TTCGTAACAC
4551  TAATTCTGTA TTAGTCATTC CTCTTGTTTG ATAGTATCCA AAAAAACGGC
4601  TATTTTTTTG CAATCTTATT TCCTGCATAT TATACAGATA ACATAATGAA
4651  AGAAAAAATC TTTTTTTTTG TTCTTCAATG ATGATTTCAA CCATTCTTTT
4701  AAACATTGAT CAATTCCTGA GCAACAACCC CATACACACT GGTTTATATA
4751  CCGCCCCTTT TACAGTTGAA GAAAGAAATA GAAATAGAAA TAGCAAACAA
4801  AAGATATGAC AGTCAACACT AAGACCTATA GTGAGAGAGC AGAAACTCAT
4851  GCCTCACCAG TAGCACAGCG ATTATTTCGA TTAATGGAAC TGAAGAAAAC
4901  CAATTTATGT GCATCAATTG ACGTTGATAC CACTAAGGAG TTCCTCGAGT
4951  TAATTGATAA ATTAGGTCCT TATGTATGCT TAATCAAGAC TCATATTGAT
5001  ATAATCAATG ATTTTTCCTA TGAATCCACT ATTGAACCAT TATTAGAACT
5051  TTCACGTAAA CATCAATTTA TGATTTTTGA AGATAGAAAA TTTGCTGATA
5101  TTGGTAATAC CGTAAAGAAA CAATATATTG GTGGAGTTTA TAAAATTAGT
5151  AGTTGGGCAG ATATTACCAA TGCTCATGGT GTCACTGGGA ATGGAGTGGT
5201  TGAAGGATTA AAACAGGGAG CTAAAGAAAC CACCACCAAC CAAGAGCCAA
5251  GAGGGTTATT GATGTTAGCT GAATTATCAT CAGTGGGATC ATTAGCATAT
5301  GGAGAATATT CTCAAAAAAC TGTTGAAATT GCTAAATCCG ATAAGGAATT
5351  TGTTATTGGA TTTATTGGCC AACGTGATAT GGGTGGCCAA GAAGAAGGAT
```

FIG. 13 (CONTINUED 4).

5401 TTGATTGGCT TATTATGACA CCTGGAGTTG GATTAGATGA TAAAGGTGAT
5451 GGATTAGGAC AACAATATAG AACTGTTGAT GAAGTTGTTA GCACTGGAAC
5501 TGATATTATC ATTGTTGGTA GAGGATTGTT TGGTAAAGGA AGAGATCCAG
5551 ATATTGAAGG TAAAAGGTAT AGAAATGCTG GTTGGAATGC TTATTTGAAA
5601 AAGACTGGCC AATTATAAAT GTGAAGGGGG AGATTTTCAC TTTATTAGAT
5651 TTGTATATAT GTAGAATAAA TAAATAAATA AGTTAAATAA ATAATTAAAT
5701 AAGGGTGGTA ATTATTACTA TTTACAATCA AAGGTGGTCC TTCTAGCTGT
5751 AATCCGGGCA GCGCAACGGA ACATTCATCA GTGTAAAAAT GGAATCAATA
5801 AAGCCCTGCG CTCATGAGCC CGAAGTGGCG AGCCCGATCT TCCCCATCGG
5851 TGATGTCGGC GATATAGGCG CCAGCAACCG CACCTGTGGC GCCGCAGCGC
5901 GCAGGGTCAG CCTGAATACG CGTTTAATGA CCAGCACAGT CGTGATGGCA
5951 AGGTCAGAAT AGCCCAAGTC GGCCGAGGGG CCTGTACAGT GAGGGAAGAT
6001 CTGATATTGA CGAAGAGGAA CCAATGTAAC GTTACACTGA AGAAAACACA
6051 CAATAAACGG GAAGAAACGG TGTAAAAGTG TGAAAATAAT TTTTGAATAT
6101 CATTTCCCTT GGTTTAATTC CAAACGAAAC GTGTTTTTTT TAGAGAATGG
6151 GAATTCTTAT TGGATGTCTA GATTGTTTGT TTACTCCAGA CTGTGCACAA
6201 AAACGTTTGG ATGGATGATC AGAAGATATT TTTAGGCTTA GCTCTAAATA
6251 TAAGAAATGA TGCTTGAAAA ACCAGACAGA AATTGAGTTT CAAAAATTGG
6301 TAATGTGAGG TATTAGTCAA CTAACCAAAT AACAATGCAA ACCGGTTGAT
6351 ACATTTCATT TTGAAAATAA TGAAACTGGA ATTGGATGAC CAGCACACAA
6401 ACACATAAAG TAATTATGGC AATTAGAAGC GAACATAGAG GAGTACTTGG
6451 CCACGAACAG AATACAAGTG GGAACACTAT TTTCTCCATT GTTTTAGTTC
6501 TGTTTTTTTG TCAGCCTAGT TTTGTGCTAT GTGTAAAAAA TATTGCCAAG
6551 AAAAAAAGCT TGTTTTGTGG CCAGTGTCCG AAAAAAATTT TGGGGAATCT
6601 TCGGATTAAT TTATGTTTTC A

FIG. 14.

MITDEQLNTI ALTFGFASII LIIIYHAIST NVHKLEDETP SSSFTRTNTT
1 50

ETTVASKKKK
51 60

FIG. 15.

MLKTLTQTLR LTGKAFPKVR PALIRTYAAF DRSKPHVNIG TIGHVDHGKT
1 50

TLTAAITKVL AEQGGANFLD YGSIDRAPEE RARGITISTA HVEYETKNRH
51 100

YAHVDCPGHA DYIKNMITGA AQMDGAIIVV AATDGQMPQT REHLLLARQV
101 150

GVQDLVVFVN KVDTIDDPEM LELVEMEMRE LLSTYGFDGD NTPVIMGSAL
151 200

MALEDKKPEI GKEAILKLLD AVDEHIPTPS RDLEQPFLLP VEDVFSISGR
201 250

GTVVTGRVER GVLKKGEEIE IVGGFDKPYK TTVTGIEMFK KELDSAMAGD
251 300

NCGVLLRGVK RDEIKRGMVL AKPGTATSHK KFLASLYILT SEEGGRSTPF
301 350

GEGYKPQCFF RTNDVTTTFS FPEGEGVDHS QMIMPGDNIE MVGELIKSCP
351 400

LEVNQRFNLR EGGKTVGTGL ITRIIE
401 426

FIG. 16.

MEVTQRTQSQ TQPTQQSPTT QTQTQSKEDQ NRICQLICST GQFGNYDLNI
1 50

NDKTIVQGKM TWYFGRDPNS DLQVASSSRI SNKHFQIWLN FNDKSLWIKD
51 100

TSTNGTHLNN SRLVKGSNYL LNQGDEIAVG VGRDEDVVRF VVVFGDKYNP
101 150

AKLPDSTNTI KDEGIYKDFI VKNETIGQGA FATVKKAIER STGESYAVKI
151 200

INRRKALNTG GGSAMAGVDR ELSILERLNH PNIVALKAFY EDMDNYYIVM
201 250

ELVPGGDLMD FVAANGAIGE DATQVITKQI LEGIAYVHNL GISHRDLKPD
251 300

NILIMQDDPI LVKITDFGLA KFSDNSTFMK TFCGTLAYVA PEVITGKYGS
301 350

SQMESQQKDN YSSLVDIWSL GCLVYVLLTS HLPFNGKNQQ QMFAKIKRGE
351 400

FHEAPLNSYD ISEDGRDFLQ CCLQVNPKLR MTAAEALKHK WLQDLYEEDS
401 450

VKSLSLSQSQ SQQSRKIDNG IHIESLSKID EDVMLRPLDS ERNRKSSKQQ
451 500

DFKVPKRVIP LSQHPATPLP MSQPKKRPYQ IDPRTNKKVD LEEPSTSKKV
501 550

KLSDSVVAED YLKLEPLANS LFQETINISK SPFSFGRNDT CDCEIDDDRL
551 600

SKLHCVITKE NDSIWLLDKS TNSCLVNNTS VGKGNKVLLR GGEILHLFFD
601 650

PLSSQHIGFK VVLVDQSSGE HKSQVEVLKQ TSEEMNIIPL ISGLSSISS
651 699

FIG. 17.

MGTSTSEALK NIKNKQRRQK VFAEIKHEKN KQRHKQRAER AKEERENPEL
1 50

REERIAANIP DTIDSKRIYD ETIAAEVEGD DEFQSYFTNL LEEPKILLTT
51 100

SANAKKPAYE FADMIMDFLP NVTFIKRKKE YTMQDMAKYC SNRDFTALLV
101 150

INEDKKKVNG ITLINLPEGP TFYFSITSIV DGKRIKGHGK AGDYLPEIVL
151 200

NNFNSRLGKT VGRLFQSIFP HKPELQGRQV ITLHNQRDYI FFRRHRYIFR
201 250

NEEKVGLQE GPQFTLKLRRM QKGVRGDVVW EHRPDMERDK KKFYL
251 295

FIG. 18.

MNSEKIIEVI IAIFLPPVAV FMKCGATTPL WINLVLCIFI WFPAILHALY
1 50

VVLKD
51

FIG. 19.

MTLGFDKFIS KVSTHRRQSE PSILEIAATN SQNKSRRLSM DNGHCYVRES
1

50

TNNHHHLNTV VDNLRQRAGS FSFISHHHNH HQNSHDNYTV DPLTSNGARI
51

100

SRSRSRSKSV GHGEAISPAY FSKNKTKDLV KQETAHIISK KLLNMLQDLD
101

150

LQNPIALKTI SQGSESKFCK IYVSNTNNCI YLPAASSTSF TYEDDENGGV
151

200

IIAEDRNDEM PTAVNNNTLS MDSINHSETD FSDSPPPPDL FSKMKSFHSP
201

250

NYLTSKIDSE CPIPHTFAVI VELTKDSLII KDLHFQFQSL TTILWPTGDA
251

300

YNRTHAKEKF TIGNMEWRTS LSDADYYINS SNSNDVKSKN LGPEDLINRT
301

350

REYKLIDIEE PNNSSNSLSD DDMDINNITS PLSTSPTSSS TSTNSTSNSL
351

400

GSDSYKAGLY VFLLPILLPE HIPASIVSIN GSLAHTLSVE CNKYTDKLNR
401

450

KSKVSASYNL PMVRTPPNIG NSIADKPIYV NRIWNDAVHY IITFPRKYVT
451

500

LGCEHMINVK LSPMVKDVVI KRIKFNVLER ITYVSKNLSR EYDYDSEDPY
501

550

CIHPVSKENK VRERVVSLYE LKTKAKQSSC GHLEAYKQEV MKCPENNLLF
551

600

SCYEVENDNN NGNGNGNGNG NKNVKQKNKD QPMIATPLDI NVSLPFLTTM
601

650

SDSLIMTSAI EEEGSDSPHT SRRGSAVSMT DNNTTPSNNN PLSPFLGAVE

FIG. 19 (CONTINUED).

651

700

TNGASINEIG DHTLFPDSNF RHIEIKHRLQ VTFRISKPDS DNKMHHYEVV
701

750

IDTPIVLLSS KCQEDSPPPY SSV
751 773

FIG. 20.

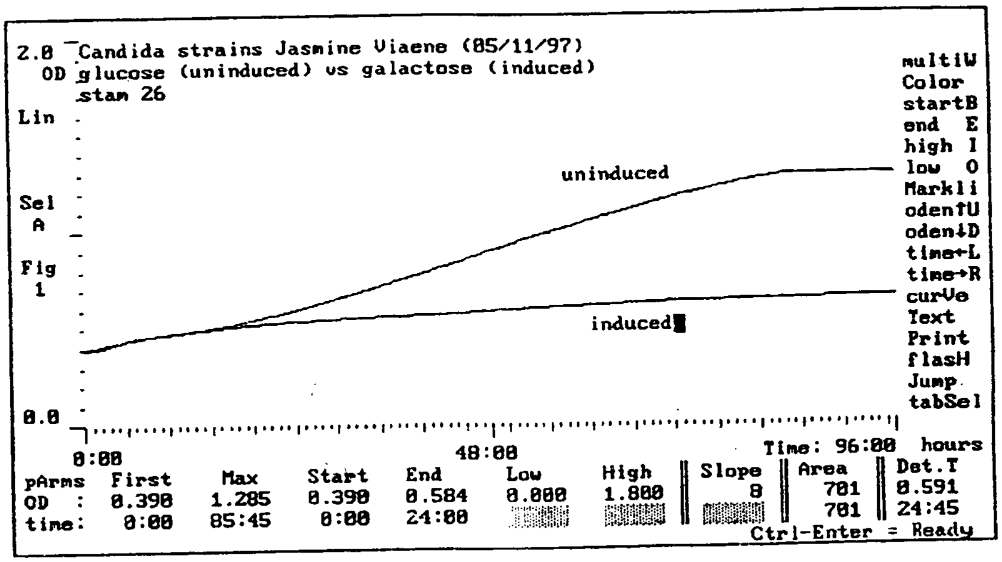
MGEGTPSLGKRHNKSHTLCNRCGRRSFHVQKKTCSSCGYPAAKMRSHNWALKAKRRRTTGTGRMAYLK
HV
TRRFKNGFQTGVAKAQTPSA

FIG. 21.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 26g
Freezer location : glycerol stocks box XXIII; C8
Growth curve(s) (Bioscreen) :

Date : 05/11/1997



Plasmid/clone name* : 26g3
Freezer location : original stocks box
Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

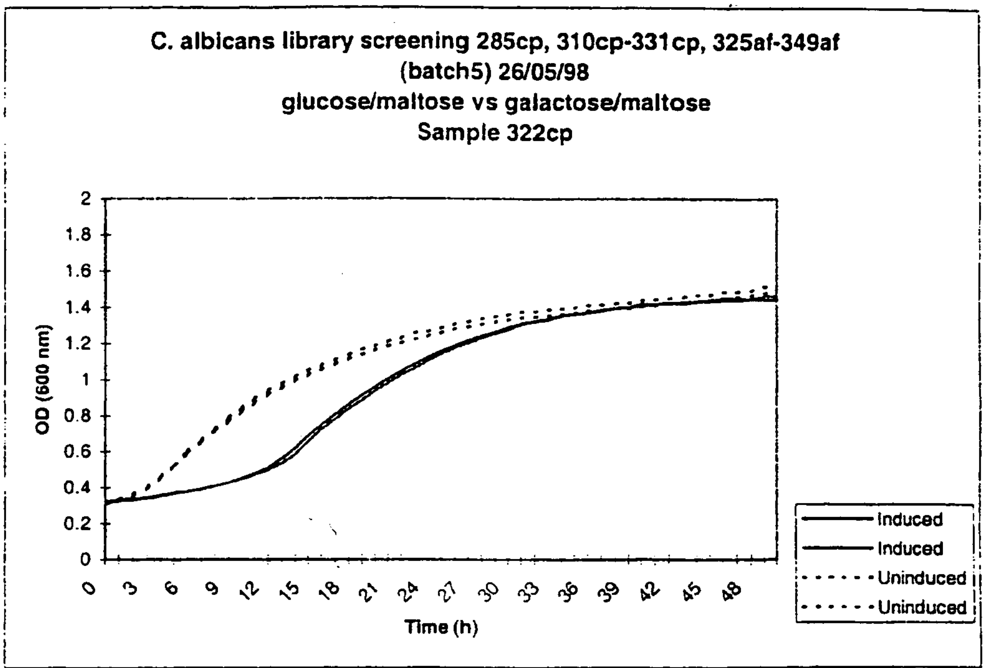
(*) as it can be found in the Candida albicans Access dbase

FIG. 22.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 322c_cp
Freezer location : glycerol stocks box XIV; D6
Growth curve(s) (Bioscreen) :

Date : 26/05/1998



Plasmid/clone name* : 322c_cp
Freezer location : original stocks box
Identifier (gene name) :

HTS screen :

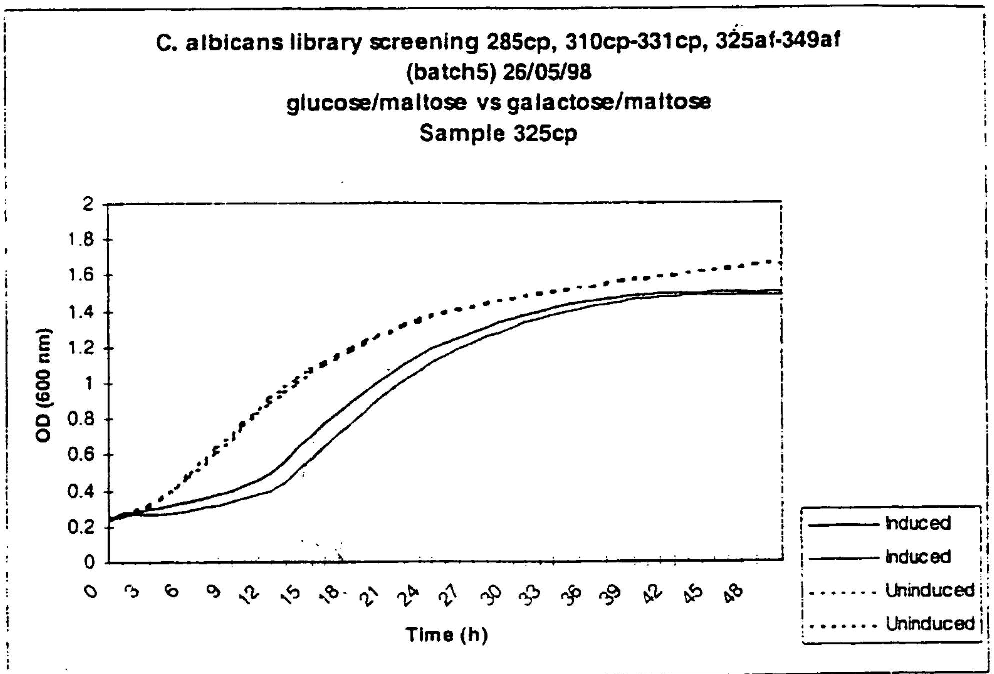
Form generated by : Inge Loonen

(*) as it can be found in the Candida albicans Access dbase

FIG. 23.

<u>Project : Identification of novel essential genes in *C. albicans*</u>

Strain no. : 325c_af
Freezer location : glycerol stocks box XIII; G4
Growth curve(s) (Bioscreen) : Date : 26/05/1998



Plasmid/clone name* : 325c_af
Freezer location : original stocks box
Identifier (gene name) :

HTS screen :

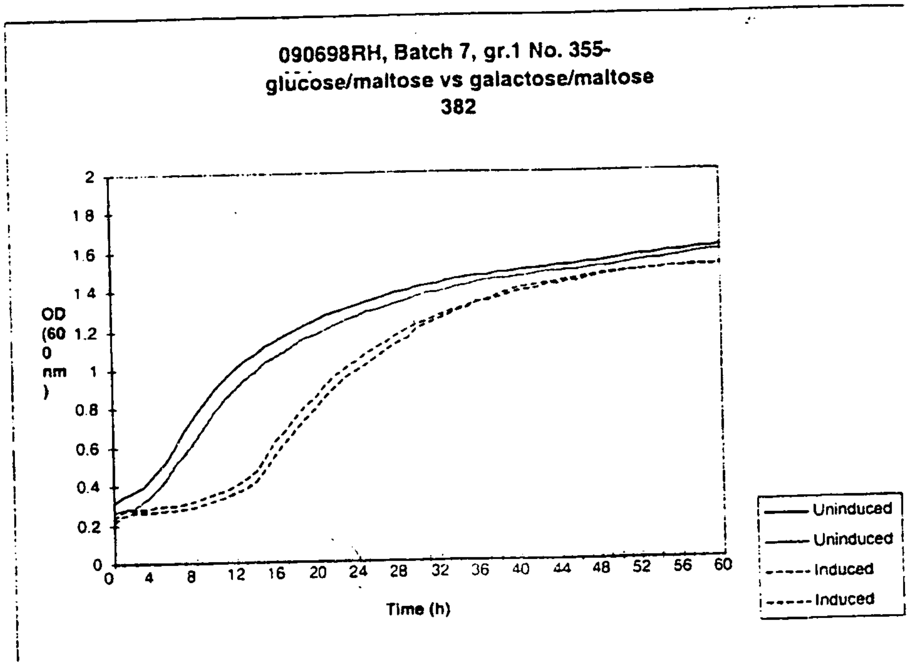
Form generated by : Inge Loonen

(*) as it can be found in the Candida albicans Access dbase

FIG. 24.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 382cp (FACS, batch 7, G1)
Freezer location : glycerol stocks box XVI; A2
Growth curve(s) (Bioscreen) : Date : 09/06/98



Plasmid/clone name* : 382cp (purified PCR product)
Freezer location : original stocks box VIII; AAH8
Identifier (gene name) : OST4

HTS screen :

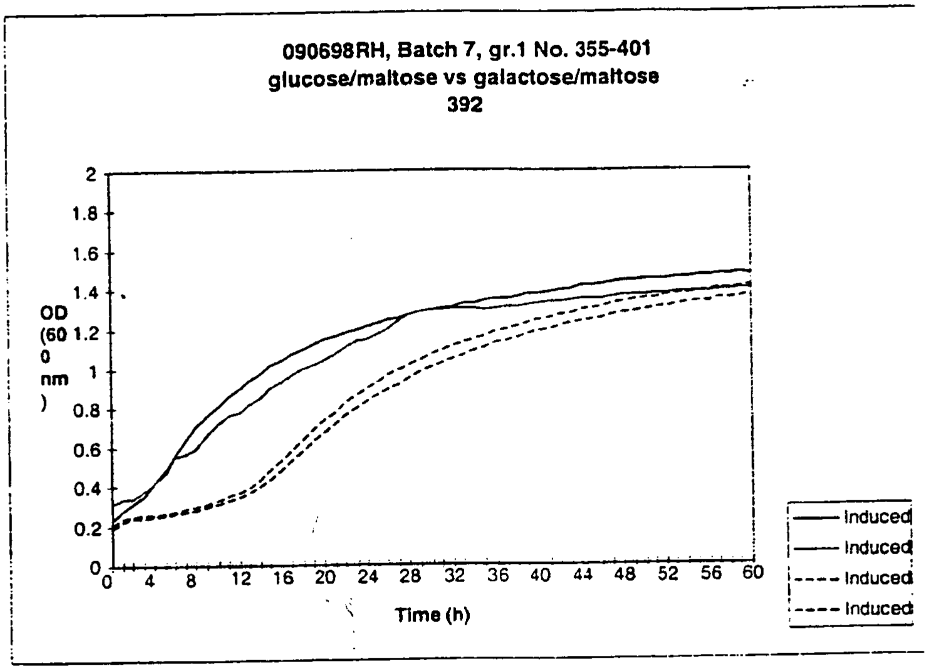
Form generated by : Inge Loonen

(*) as it can be found in the Candida albicans Access dbase

FIG. 25.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 392cp (FACS, batch 7, G1)
Freezer location : glycerol stocks box XVI; B3
Growth curve(s) (Bioscreen) : Date : 09/06/98



Plasmid/clone name* : 392cp (purified PCR product)
Freezer location : original stocks box VIII; AAH2
Identifier (gene name) : TUF1

HTS screen :

Form generated by : Inge Loonen

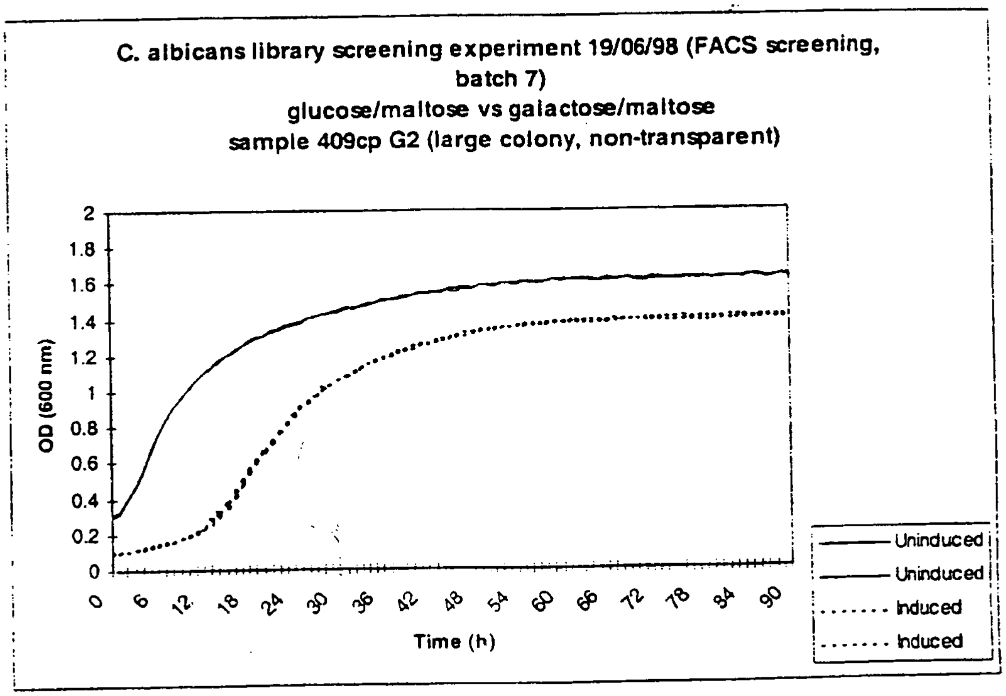
(*) as it can be found in the Canäida albicans Access dbase

FIG. 26.

<u>Project : Identification of novel essential genes in *C. albicans*</u>

Strain no. : 409c_cp
Freezer location : glycerol stocks box XVI; C9
Growth curve(s) (Bioscreen) :

Date : 19/06/1998



Plasmid/clone name* : 409c_cp
Freezer location : original stocks box
Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

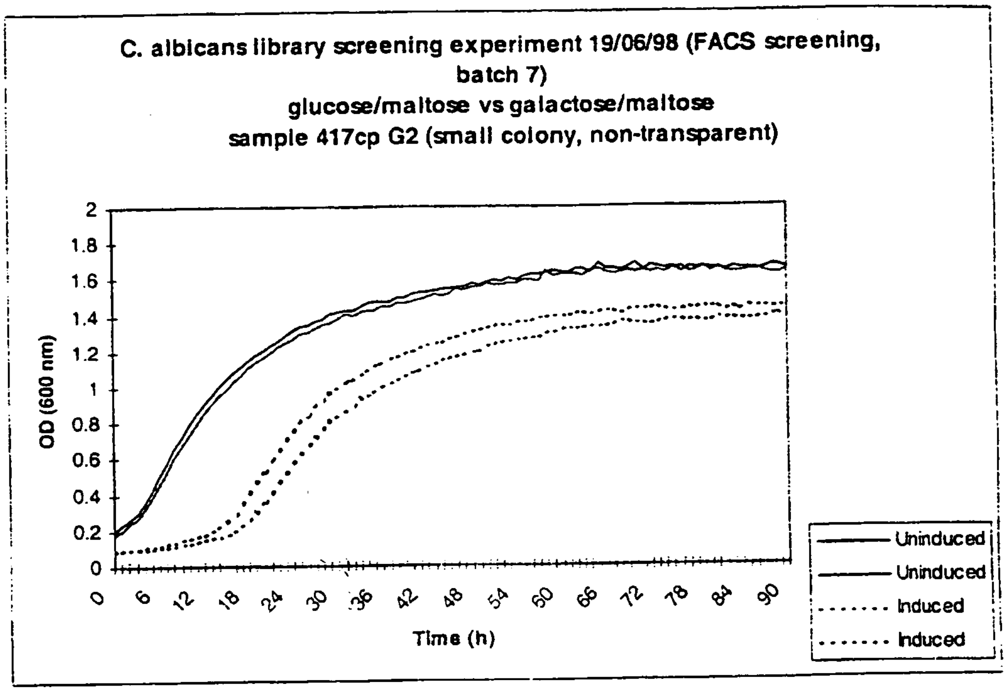
*(*) as it can be found in the Candida albicans Access dbase*

FIG. 27.

Project : Identification of novel essential genes in *C. albicans*

Strain no. : 417c_cpG2
Freezer location : glycerol stocks box XVI; D8
Growth curve(s) (Bioscreen) :

Date : 19/06/1998



Plasmid/clone name* : 417c_cpG2L
Freezer location : original stocks box
Identifier (gene name) :

HTS screen :

Form generated by : Inge Loonen

(*) as it can be found in the Candida albicans Access dbase

FIG. 28.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : 325caf
Disruptant strain :
Host strain :
Freezer location :

Disruption plasmid name* :
Freezer location :

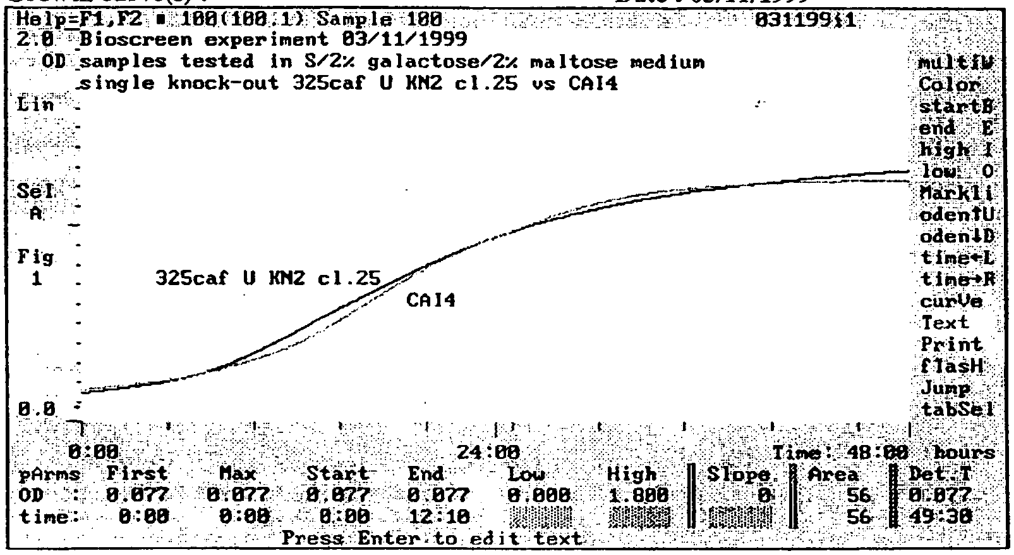
Knock-out (single/double):
Lab book ref. :

Southern results :

PCR results :

FIG. 28(CONTINUED 1).

Growth curve(s) : Date : 03/11/1999



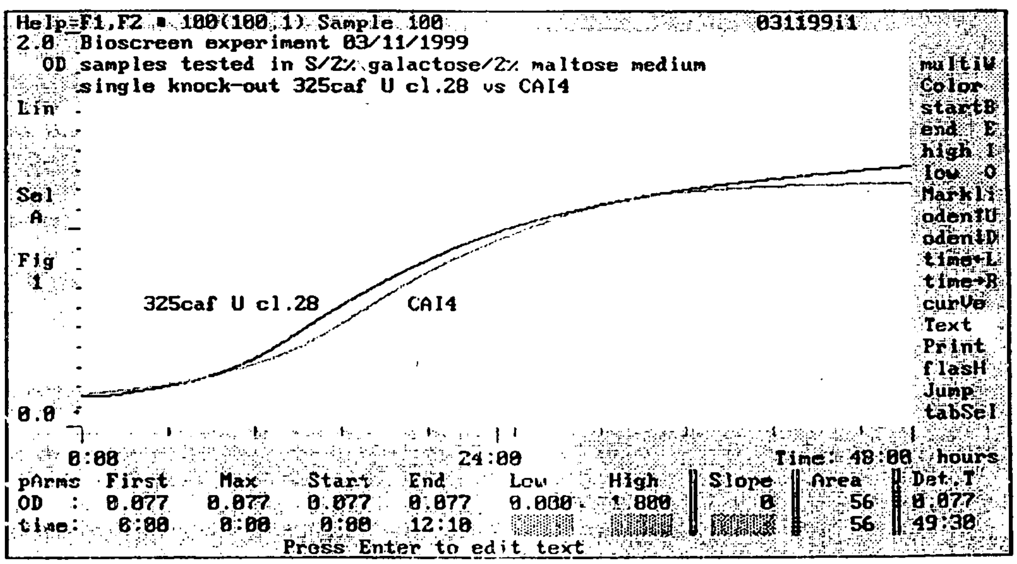
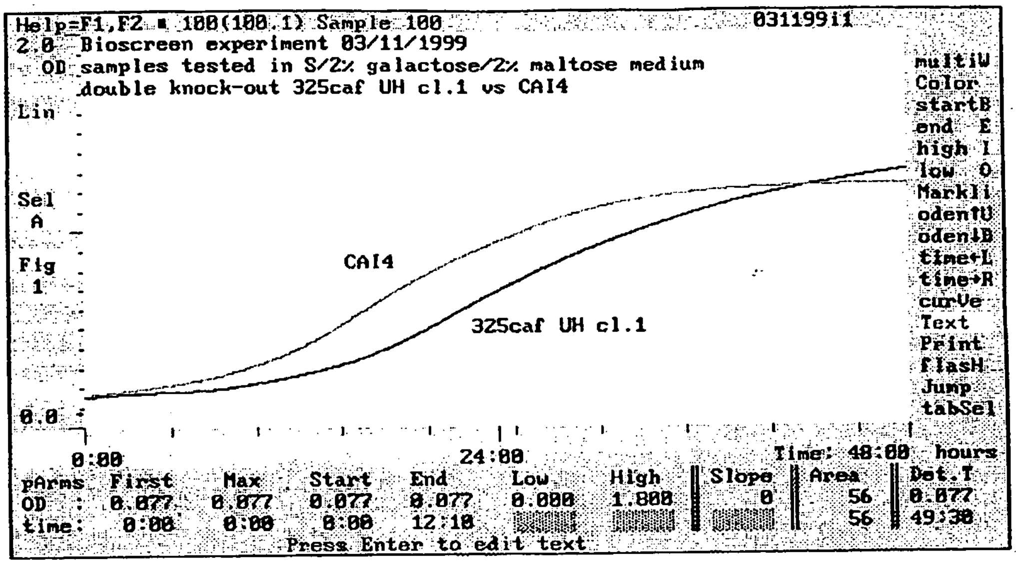
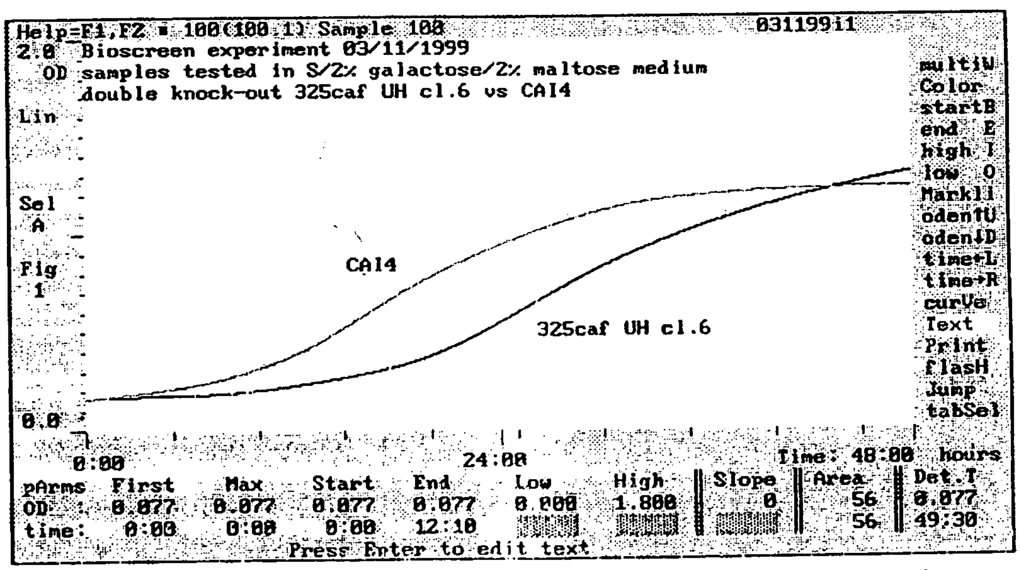


FIG. 28 (CONTINUED 2)





HTS screen :

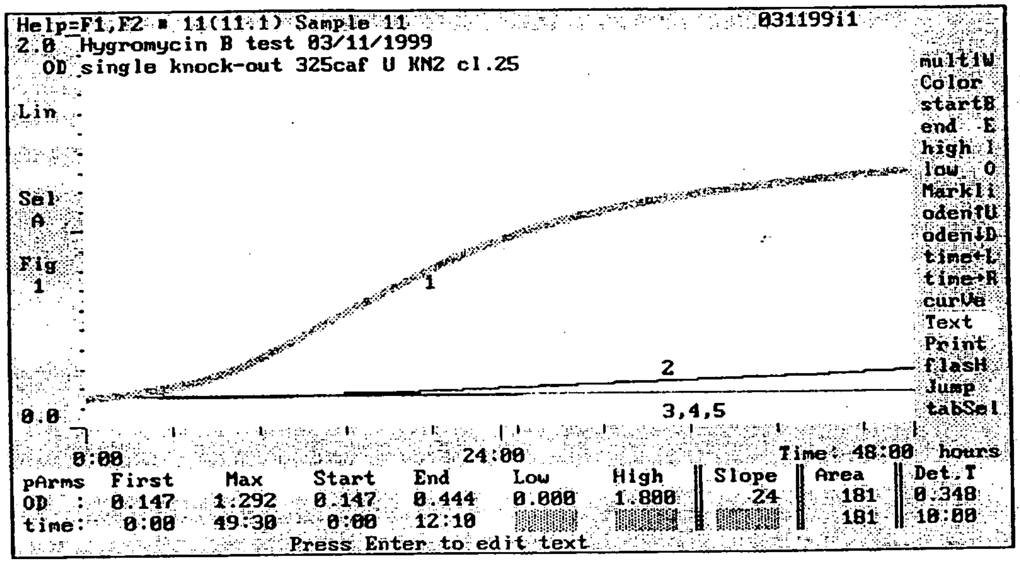
Bioscreen test of 325caf knock-out and WT growth in presence of hygromycin B

dilutions prepared

Stock solution of 53 mg/ml was prepared for hygromycin B.
From this solution dilutions were prepared of:
4000μg/ml, 3000μg/ml, 2000μg/ml and 1000μg/ml

Growth curves for 325cafgK knock-out and WT in the presence of hygromycin B

FIG. 28 (CONTINUED 3).



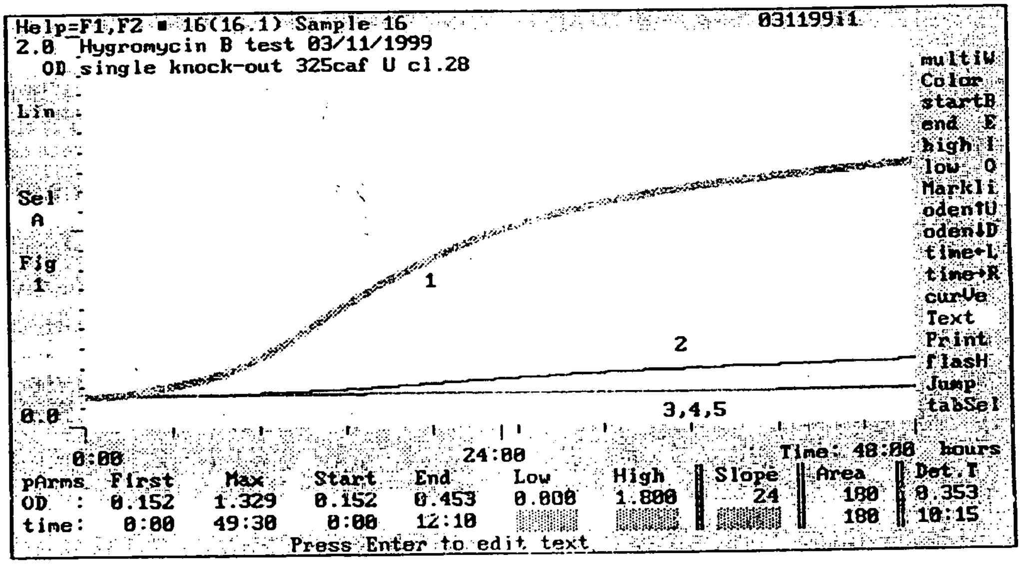
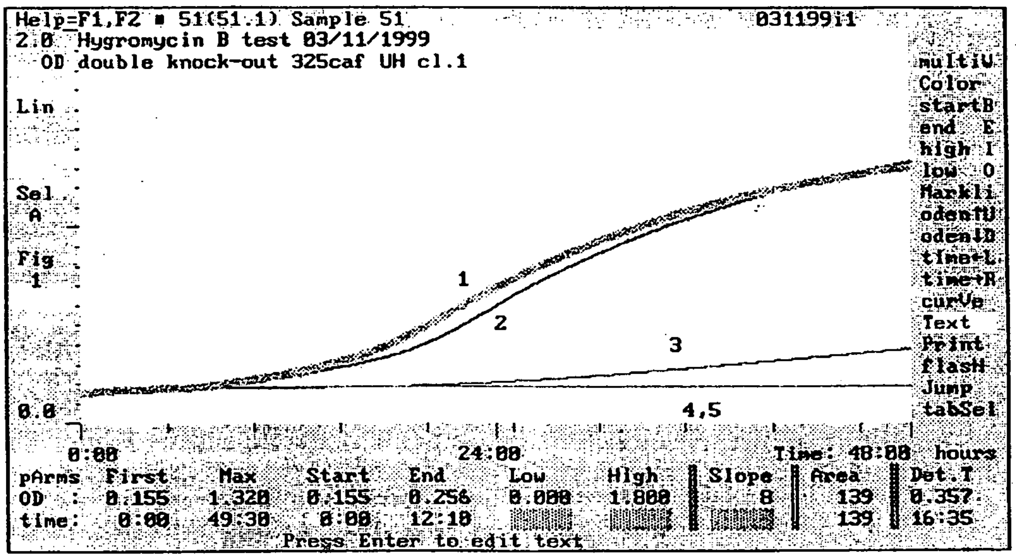


FIG. 28 (CONTINUED 4)



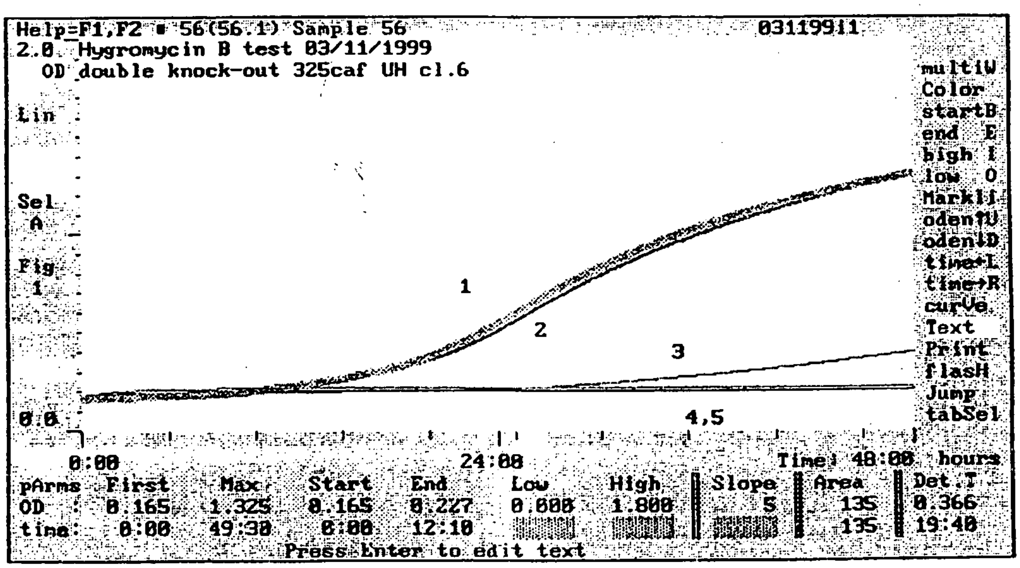
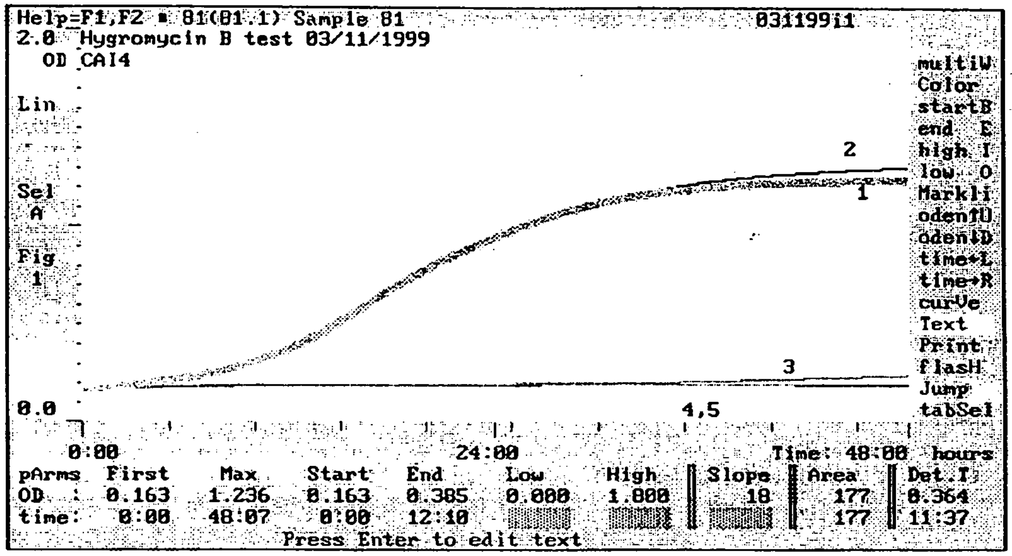


FIG. 28 (CONTINUED 5).



Legend:

1: S/2% gal/2% mal medium containing 0 μg/ml Hygromycin B
2: S/2% gal/2% mal medium containing 1000 μg/ml Hygromycin B
3: S/2% gal/2% mal medium containing 2000 μg/ml Hygromycin B
4: S/2% gal/2% mal medium containing 3000 μg/ml Hygromycin B
5: S/2% gal/2% mal medium containing 4000 μg/ml Hygromycin B

Form generated by :

(*) as it can be found in the Plasmid Access dbase

FIG. 29.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) :	322c_cp
Disruptant strain :	322c_cp (in progress)
Host strain :	CAI4NG
Freezer location :	Knockout strain, box, pos.
Disruption plasmid name* :	322c_cpURAcass.(inv)/pCR2.1(inv)
Freezer location :	-
Knock-out (single/double):	single (in progress)
Lab book ref. :	Labbook 104 of Ronald de Hoogt

Southern results :

PCR results :

FIG. 30.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : 417c_cpG2
Disruptant strain :
Host strain :
Freezer location :

Disruption plasmid name* :
Freezer location :

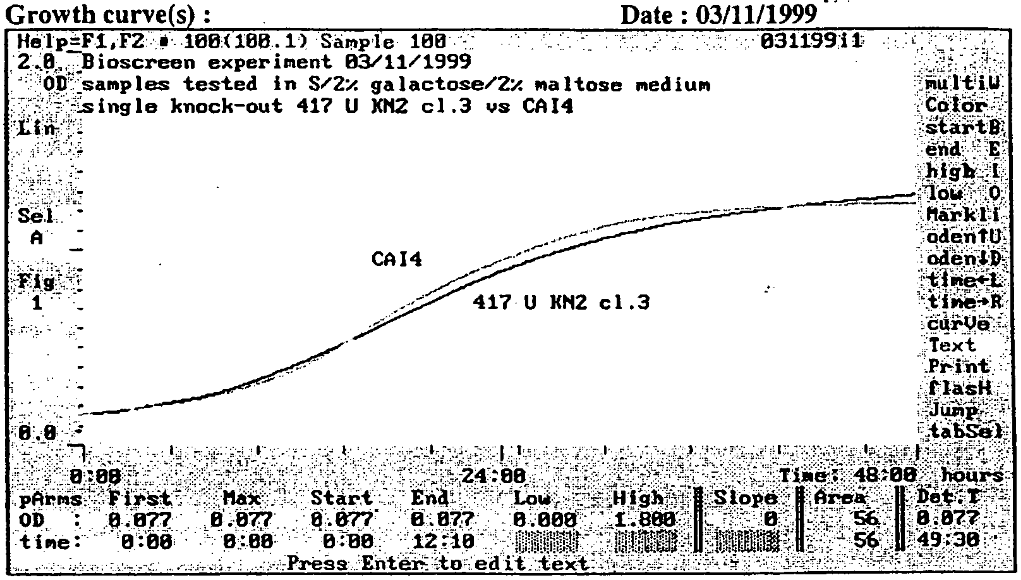
Knock-out (single/double):
Lab book ref. :

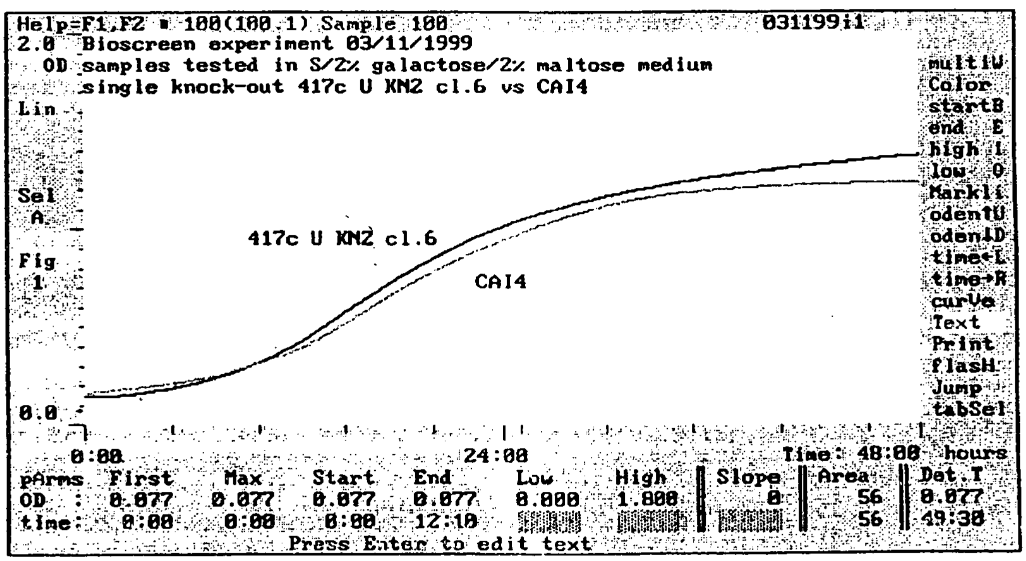
Southern results :

PCR results :

FIG. 30 (CONTINUED 1).

Growth curve(s) : Date : 03/11/1999





HTS screen :

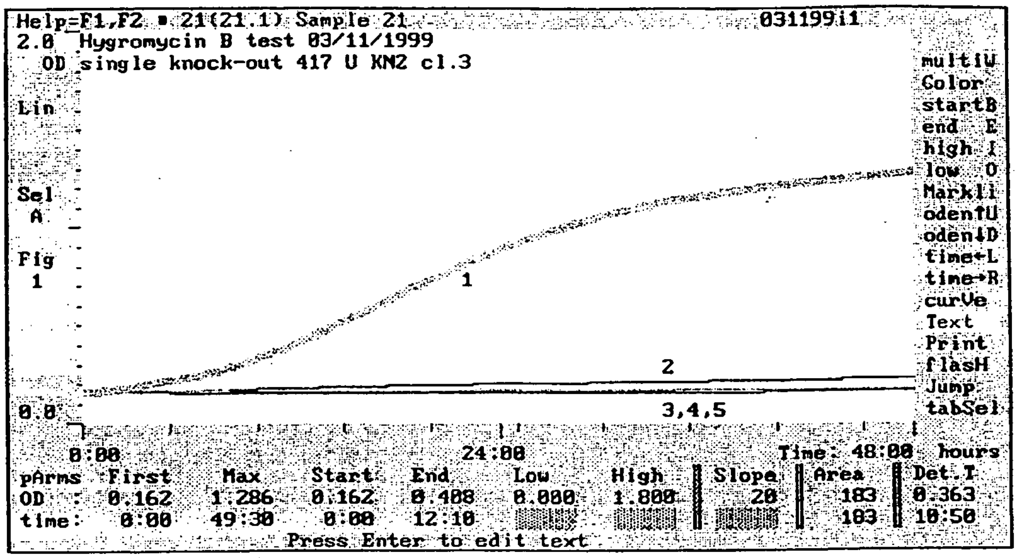
Bioscreen test of 417c_cp knock-out and WT growth in presence of hygromycin B

dilutions prepared

Stock solution of 53 mg/ml was prepared for hygromycin B.
From this solution dilutions were prepared of:
4000µg/ml, 3000µg/ml, 2000µg/ml and 1000µg/ml

Growth curves for 417c_cp knock-out and WT in the presence of hygromycin B

FIG. 30 (CONTINUED 2).



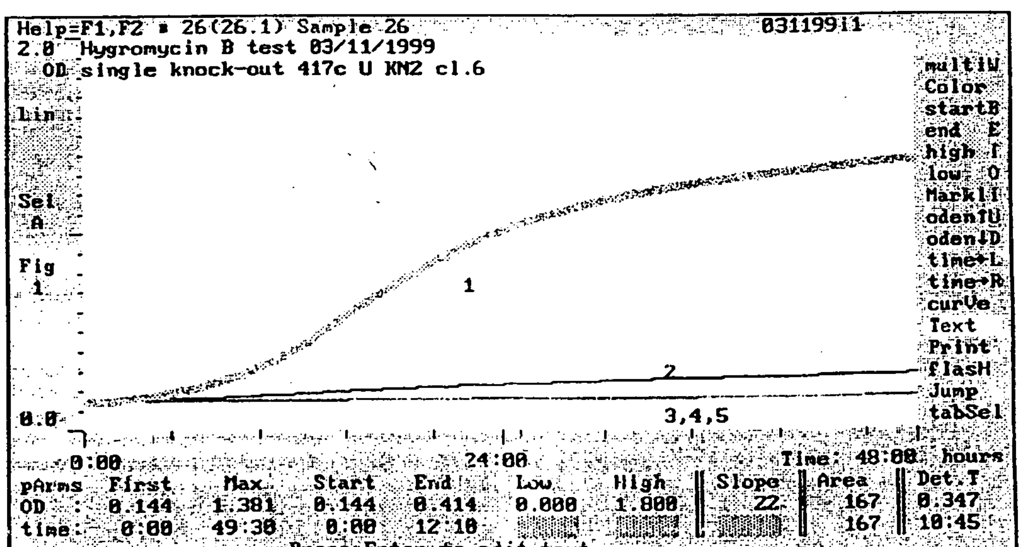
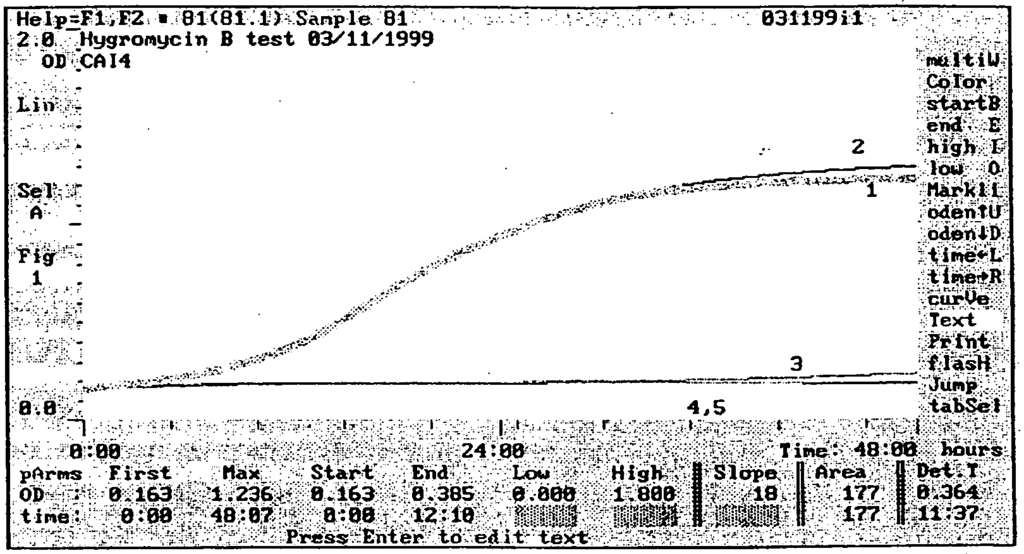


FIG. 30 (CONTINUED 3).



Legend:

1: S/2% gal/2% mal medium containing 0 µg/ml Hygromycin B
2: S/2% gal/2% mal medium containing 1000 µg/ml Hygromycin B
3: S/2% gal/2% mal medium containing 2000 µg/ml Hygromycin B
4: S/2% gal/2% mal medium containing 3000 µg/ml Hygromycin B
5: S/2% gal/2% mal medium containing 4000 µg/ml Hygromycin B

Form generated by :

(*) as it can be found in the Plasmid Access dbase

FIG. 31.

Project : Identification of novel essential genes in *C. albicans*

Identifier (gene name) : TUF1

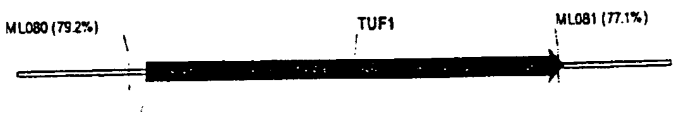
Disruptant strain : TUF1SAKO 7 **Host strain :** CAI4/NG

Freezer location : Strain collection Roland Contreras. YA132

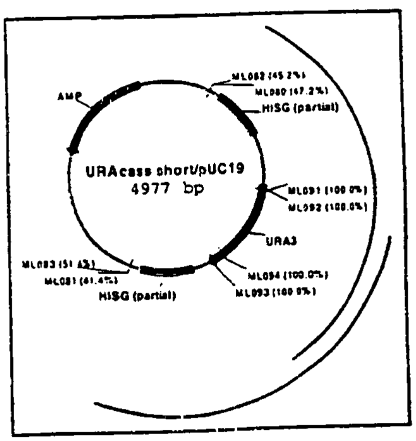
Disruption plasmid name* : Not applicable: short terminal homology (STH) PCR with overlapping fragments (split marker).

ML080	acataatcaagtgaatttacttacatcatttattgtggaaatttctgaatGTGCTGGAATTCGCCCTTTATG
ML081	tcacctatatatacctctttttctttttattattcacagtgcacattctgtCCGGCTCGTATGTTGTG TGG
ML094	CCAGTGCTAACAACTTCATCAACAGTT
ML092	GCCTCACCAGTAGCACAACG

Uppercase sequences are segments that anneal to the template DNA URAcass short/pUC19: the lowercase sequences are 50 nt uptstream (ML080), resp. downstream (ML081) of the target ORF.

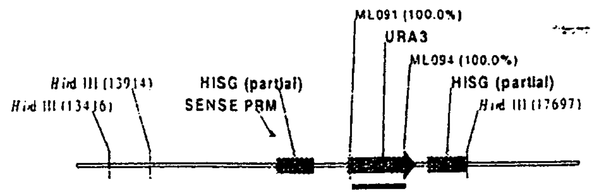


Amplification of STH fragments for TUF1 (ML080/ML094 ⇒ TUF1_STH-5'URA3 and ML081/ML092 ⇒ TUF1_STH-3'URA3). TUF1_STH-5'URA3 has a 50 bp terminal homology region upstream of the TUF1 ORF and a 3' incomplete URA3 marker; while TUF1_STH-3'URA3 has a 50 bp terminal homology region downstream of the TUF1 ORF and a 5' incomplete URA3 marker. *In vivo*, only an intact URAblast cassette can be formed when recombination occurs between the overlapping truncated URA3 sequences of the respective STH fragments.



Southern results: FIG. 31 (CONTINUED 1).

Presentation of disrupted allele

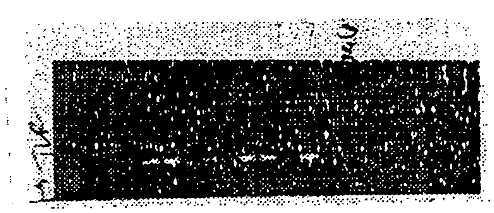


Fragment of TUF1 SAKO

*Hind*III digest
URA3 probe
Expected band: 3783 bp

PCR results:

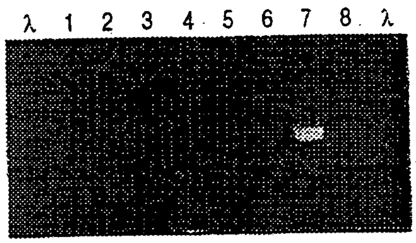
- PCR analysis was performed using the primer combination ML091/ML093 (see figure), amplifying a URA3 fragment.. **Band of 755 bp points to correct homologous recombination of URA3 overlapping fragments.** TUF1 SAKO 7 is clearly positive (SAKO stands for single allele knock out).



lane 1: λ *Pst*I
lane 2 to 9: potential TUF1 SAKOs 1 to 8
lane 10: Uracass short/pUC19 (positive control)
lane 11: water
lane 12: CAI4

FIG. 31(CONTINUED 2).

> To check correct integration into the genome, PCR was performed with primer sets ML090/ML097. For TUF1 SAKO 7 a clear signal was obtained of the correct length of 1825 bp.

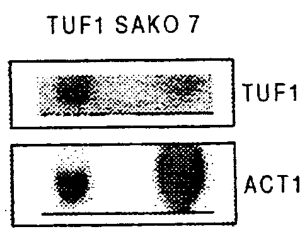


λ: λ *PstI*

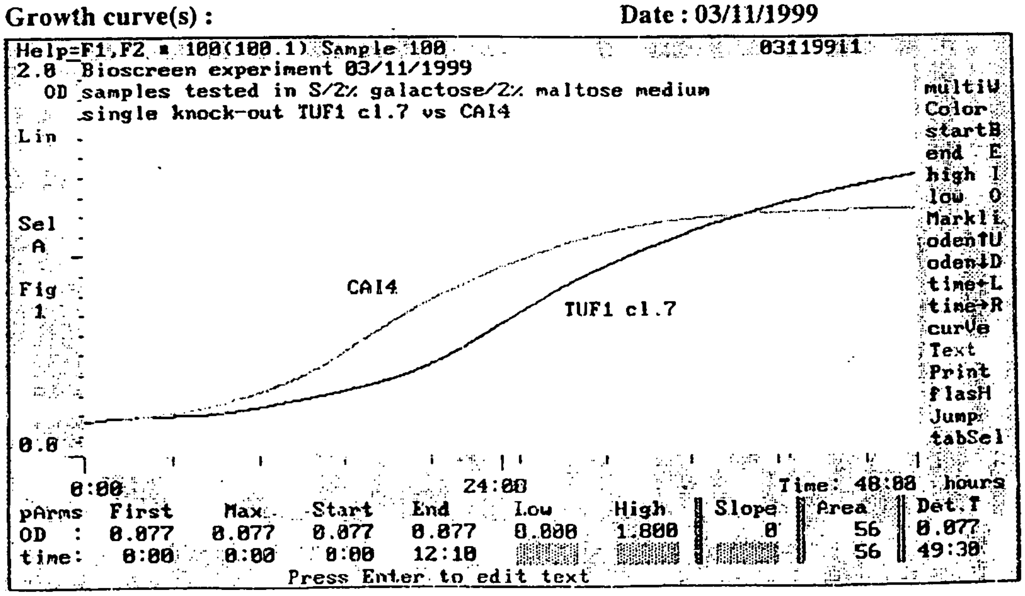
1 to 8: potential TUF1 SAKOs 1 to 8

Northern analysis:

88% inhibition



Growth curve(s) : **Date : 03/11/1999**



HTS screen :

FIG. 31 (CONTINUED 3).

Bioscreen test of TUF1 knock-out clone 7 and WT growth in presence of hygromycin B

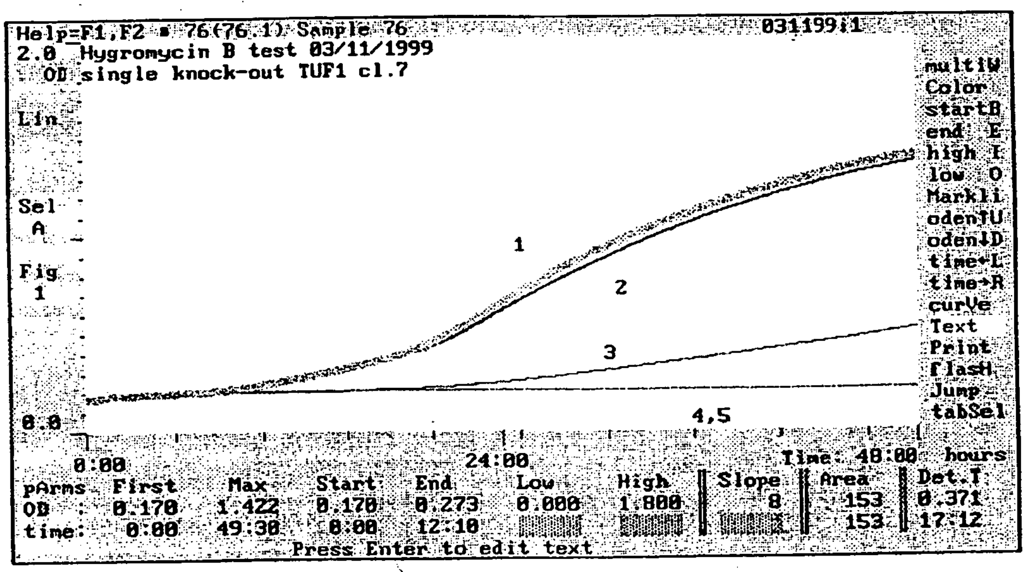
dilutions prepared

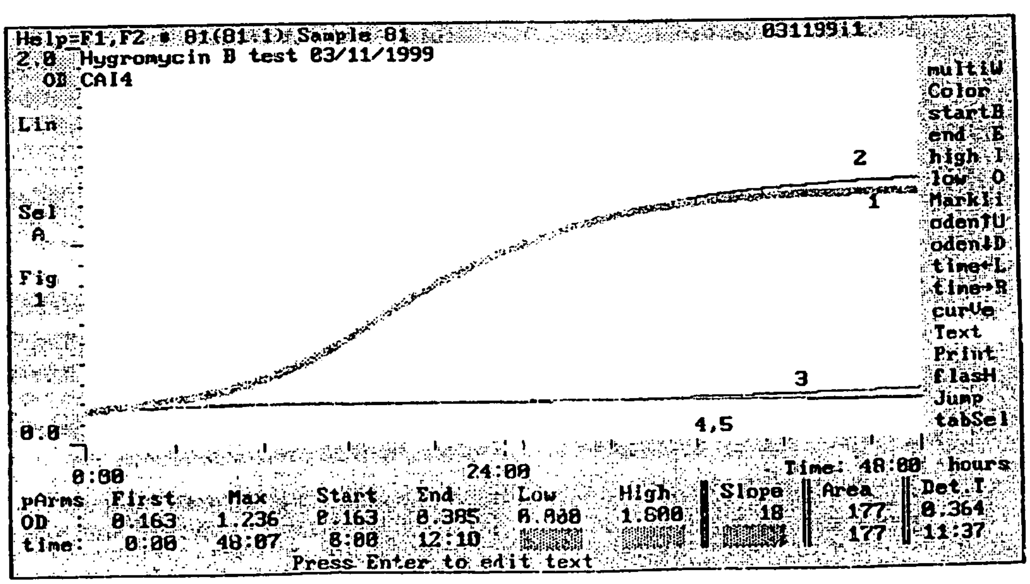
Stock solution of 53 mg/ml was prepared for hygromycin B.

From this solution dilutions were prepared of:

4000μg/ml, 3000μg/ml, 2000μg/ml and 1000μg/ml

Growth curves for TUF1 knock-out and WT in the presence of hygromycin B





Legend:

FIG. 31 (CONTINUED 4).

1: S/2% gal/2% mal medium containing 0 μg/ml Hygromycin B
2: S/2% gal/2% mal medium containing 1000 μg/ml Hygromycin B
3: S/2% gal/2% mal medium containing 2000 μg/ml Hygromycin B
4: S/2% gal/2% mal medium containing 3000 μg/ml Hygromycin B
5: S/2% gal/2% mal medium containing 4000 μg/ml Hygromycin B

SEQUENCE LISTING

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<120> Drug Targets in Candida Albicans

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ttttgaaaca aacctcagaa gaaatgaata ttattccact tatttctggt ttaagtagta 2160
taagttcata gatttagcat atatacaagc atttcctata gaaacaaagg ttcattaatt 2220
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atc 2283

<210> 4
<211> 826
<212> DNA
<213> Candida albicans

<400> 4
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gaaagaatca ccatagttgc aagatttgat agatgttaaa atgttcacgc aggcgaaaga 180
tgtaacatct cttaaagtaa gaagaatatg gacatgaata aaaatagata gcactatttt 240
ggaacttgtt gaagatatta aaatagaatg ggatttcaac atagatattc aaagtaacga 300
aacctcacaa tcaaataaaa acaacagtaa tactaacaat tcaattttta tttttataga 360
gggtactcca tctttaggta aacgtcacaa caaatctcac accttatgta acagatgtgg 420
ccgtcgttca ttccacgtcc aaaagaagac ctgttcttct tgtggttacc cagctgctaa 480
aatgagatct cacaactggg ccttaaaagc caaaagaaga agaactactg gtaccggtag 540
aatggcttac ttgaaacacg ttaccagaag attcaagaac ggtttccaaa ctggtgttgc 600
taaagctcaa accccttccg cttaaactaa ttactgaagt tattggtcat gcattagtca 660
ttattcatta aagtcatgtt aagcatagca aaggaagaat tggttagatt cttgtttaaa 720
atgtaatgac tatttaatat ctgtttaaat aagaggttta gtctttattt ttttacgtat 780
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<210> 5
<211> 978
<212> DNA
<213> Candida albicans

<400> 5
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gctaaggaag aaagagaaaa ccccgaatta agagaggaaa gaatagcagc taatatccca 180
gatactatag atagcaaacg tatttatgat gagactatag ctgctgaagt tgaaggagat 240
gacgagtttc agtcatattt cactaacttg ttggaagaac caaagatttt gttgacaaca 300
agtgccaatg ctaaaaaacc ggcctatgaa tttgcagaca tgatcatgga ctttttaccg 360
aatgtgacat ttatcaaaag gaagaaggaa tatacaatgc aagatatggc caaatattgc 420
tcgaatagag acttcactgc attgcttgtc atcaacgaag acaagaagaa ggtcaatggt 480

ataacgctca tcaatttacc tgaagggcca acattttatt tttcgattac atcaatagtt 540
gatgggaaaa gaattaaggg acacgggaaa gctggtgatt atttacctga gattgtattg 600
aataatttca attcaagatt gggtaaaact gtgggaagac tatttcaaag tattttccct 660
cataaacctg aacttcaagg aagacaagtg attactttgc acaatcaacg tgattatatt 720
tttttcagaa gacatagata tattttcaga aatgaggaaa aggttggatt gcaggaattg 780
ggtccgcagt ttacattaaa gctaagaaga atgcaaaagg gagtacgtgg tgatgttgtt 840
tgggaacaca gaccagatat ggaaagagat aagaagaagt tttatttata agcgggtgta 900
taaaggtagt agtagtgcgt ttataagtat gtgtgtgtgt ttatgcatag atgtgtaaag 960
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<210> 6
<211> 619
<212> DNA
<213> Candida albicans

<400> 6
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ttaattatga attctgaaaa gattattgaa gttatcattg ctattttctt accaccagta 120
gctgtgttta tgaaatgtgg tgccactacc ccattatgga ttaacttggt attatgtatc 180
tttatttggt tccctgctat cttacatgcc ttatacgttg tgttgaaaga ttaaacaaac 240
accagagatt tactgcttga tgaattgatt actccaaaga gttgtgacta gttcccagtg 300
tgtttttttt gccttccaac tttcttttac atttttccat tactaccact gtcttccccc 360
ctattttgca gagttttcaa aatttatcca aaacatgtta gtcattaaac catattatta 420
taattattct tttttgtatt tttttccctt aaaacacgtt aatttattaa tcgtttcgtt 480
gtttggtatt ttattttttt gtatttatca attggaatat atatctatac atgaatttat 540
tatccattgt accaattgtt aaaacatttt gttagttttt tgttactagt ataaaannat 600
aataaaagtt tanttcaac 619

<210> 7
<211> 2319
<212> DNA
<213> Candida albicans

<400> 7
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gataatggtc attgttatgt tcgtgaatca actaataatc atcatcattt aaataccgtc 180
gttgataatt tacgacagcg tgcgggatcg ttttcattta tttcacatca ccataatcac 240
catcagaata gtcacgataa ttatactgtc gatcccctta catcaaacgg agcacgaatt 300
tcccgatcac gttcacgttc caaatcagtt gggcacggag aagcaatatc accagcgtat 360
ttttccaaga ataaaaccaa agatttagtg aaacaggaaa cagcacatat cattctgaag 420
aaattactca acatgttaca agatttggat ttacaaaacc ctattgcatt gaaaacaata 480
tcacaaggtt cagaatcaaa gttttgtaaa atctacgtgt ctaacactaa taattgtatt 540
tacttaccag cagcaagttc aacaagtttc acttatgaag atgatgaaaa tggcggcgtt 600
ataattgctg aagatagaaa tgatgaaatg ccaacagcag ttaataacaa tactttgtca 660
atggatagta taaatcattc agagactgat ttcctggatt ctccaccacc tccagattta 720
ttttctaaaa tgaaatcatt ccattcacca aattacttga cttcaaaaat cgattctgaa 780
tgtccaattc cacatacatt tgccgtgatt gttgaatcaa ccaaggactc tttgattatt 840

aaagatcttc atttccaatt tcagtcatta actaccattt tatggccaac tggggatgca 900
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ttaagcgacg ccgactatta tatcaatagt tctaattcca acgatgttaa gctgaaaaac 1020
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ccaaacaatt catcaaacag tttactggat gatgacatgg atattaataa tattacgtcg 1140
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ggttcagatt catataaagc tggtctttat gtatttttat taccaatctt attgccagaa 1260
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cctatggtcc gtactccacc aaacattggt aattccattg ctgataagcc aatttatgtt 1440
aataggattt ggaatgatgc cgtacattat attataactt tcccccgcaa atatgttact 1500
ttgggttgtg aacacatgat aaatgtgaaa ttactgccca tggtgaaaga tgtggttatc 1560
aagcgtatta aatttaatgt attggagaga ataacttatg tttccaaaaa tttatcacga 1620
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tcttgttatg aggttgaaaa tgataataat aacggcaacg gcaacggcaa cggcaacgga 1860
aacaagaacg ttaaacaaaa gaataaagat caaccaatga ttgctacacc tttagatatc 1920
aatgtttctt taccattttt aactactatg tctgatagtt taattatgac atcagccata 1980
gaagaagaag gttcagatct gcctcataca tcaagaagag ggtcggcagt gagtatgact 2040
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actaatggtg ctagtataaa tgaaattggt gatcatacat tattccctga ttctaatttt 2160
cgacatattg aaattaaaca tcgattacaa gttacattta ggatttctaa accggatctg 2220
gataataaaa tgcatcatta tgaagtggtt attgataccc ccatcgtttt acttagttca 2280
aaatgtcaag aagattctcc tcctccttat agttctgta 2319

<210> 8
<211> 255
<212> DNA
<213> Candida albicans

<400> 8
aacgttcgtg caaaaggcta tactggtgat atccacgcag atgaagagca agtttaatca 60
actctttgtc aattaatgct gtacttgttt tcattttatt tgctggcatt taaagaatac 120
ccatagttca gaaaataaaa ttgaaaaatt taaaaaaaaa cgcaatatca ttcatttttt 180
ttgttttttt gacaataata ttaatatgta gttaccaatg tttttagatt ttatatgttt 240
tgaaaaaata gtttg 255

<210> 9
<211> 119
<212> DNA
<213> Candida albicans

<400> 9
aaccttacaa tcattatacc aactatcaaa atcataagac tcttnaactt ctgtttttga 60
tagttggtat aatgatttat gtattatctt aattcattat tattagtttc ggtcacaaa 119

<210> 10

<211> 60
<212> PRT
<213> Candida albicans

<400> 10
Met Ile Thr Asp Glu Gln Leu Asn Thr Ile Ala Leu Thr Phe Gly Phe
1 5 10 15

Ala Ser Ile Ile Leu Ile Ile Ile Tyr His Ala Ile Ser Thr Asn Val
20 25 30

His Lys Leu Glu Asp Glu Thr Pro Ser Ser Ser Phe Thr Arg Thr Asn
35 40 45

Thr Thr Glu Thr Thr Val Ala Ser Lys Lys Lys Lys
50 55 60

<210> 11
<211> 426
<212> PRT
<213> Candida albicans

<400> 11
Met Leu Lys Thr Leu Thr Gln Thr Leu Arg Leu Thr Gly Lys Ala Phe
1 5 10 15

Pro Lys Val Arg Pro Ala Leu Ile Arg Thr Tyr Ala Ala Phe Asp Arg
20 25 30

Ser Lys Pro His Val Asn Ile Gly Thr Ile Gly His Val Asp His Gly
35 40 45

Lys Thr Thr Leu Thr Ala Ala Ile Thr Lys Val Leu Ala Glu Gln Gly
50 55 60

Gly Ala Asn Phe Leu Asp Tyr Gly Ser Ile Asp Arg Ala Pro Glu Glu
65 70 75 80

Arg Ala Arg Gly Ile Thr Ile Ser Thr Ala His Val Glu Tyr Glu Thr
85 90 95

Lys Asn Arg His Tyr Ala His Val Asp Cys Pro Gly His Ala Asp Tyr
100 105 110

Ile Lys Asn Met Ile Thr Gly Ala Ala Gln Met Asp Gly Ala Ile Ile
115 120 125

Val Val Ala Ala Thr Asp Gly Gln Met Pro Gln Thr Arg Glu His Leu
130 135 140

Leu Leu Ala Arg Gln Val Gly Val Gln Asp Leu Val Val Phe Val Asn
145 150 155 160

Lys Val Asp Thr Ile Asp Asp Pro Glu Met Leu Glu Leu Val Glu Met
165 170 175

Glu Met Arg Glu Leu Leu Ser Thr Tyr Gly Phe Asp Gly Asp Asn Thr
180 185 190

Pro Val Ile Met Gly Ser Ala Leu Met Ala Leu Glu Asp Lys Lys Pro
195 200 205

Glu Ile Gly Lys Glu Ala Ile Leu Lys Leu Leu Asp Ala Val Asp Glu
210 215 220

His Ile Pro Thr Pro Ser Arg Asp Leu Glu Gln Pro Phe Leu Leu Pro
225 230 235 240

Val Glu Asp Val Phe Ser Ile Ser Gly Arg Gly Thr Val Val Thr Gly
245 250 255

Arg Val Glu Arg Gly Val Leu Lys Lys Gly Glu Glu Ile Glu Ile Val
260 265 270

Gly Gly Phe Asp Lys Pro Tyr Lys Thr Thr Val Thr Gly Ile Glu Met
275 280 285

Phe Lys Lys Glu Leu Asp Ser Ala Met Ala Gly Asp Asn Cys Gly Val
290 295 300

Leu Leu Arg Gly Val Lys Arg Asp Glu Ile Lys Arg Gly Met Val Leu
305 310 315 320

Ala Lys Pro Gly Thr Ala Thr Ser His Lys Lys Phe Leu Ala Ser Leu
325 330 335

Tyr Ile Leu Thr Ser Glu Glu Gly Gly Arg Ser Thr Pro Phe Gly Glu
340 345 350

Gly Tyr Lys Pro Gln Cys Phe Phe Arg Thr Asn Asp Val Thr Thr Thr
355 360 365

Phe Ser Phe Pro Glu Gly Glu Gly Val Asp His Ser Gln Met Ile Met
370 375 380

Pro Gly Asp Asn Ile Glu Met Val Gly Glu Leu Ile Lys Ser Cys Pro
385 390 395 400

Leu Glu Val Asn Gln Arg Phe Asn Leu Arg Glu Gly Gly Lys Thr Val
405 410 415

Gly Thr Gly Leu Ile Thr Arg Ile Ile Glu
420 425

<210> 12
<211> 699
<212> PRT
<213> Candida albicans

<400> 12
Met Glu Val Thr Gln Arg Thr Gln Ser Gln Thr Gln Pro Thr Gln Gln
1 5 10 15

Ser Pro Thr Thr Gln Thr Gln Thr Gln Ser Lys Glu Asp Gln Asn Arg
20 25 30

Ile Cys Gln Leu Ile Cys Ser Thr Gly Gln Phe Gly Asn Tyr Asp Leu
35 40 45

Asn Ile Asn Asp Lys Thr Ile Val Gln Gly Lys Met Thr Trp Tyr Phe
50 55 60

Gly Arg Asp Pro Asn Ser Asp Leu Gln Val Ala Ser Ser Ser Arg Ile
65 70 75 80

Ser Asn Lys His Phe Gln Ile Trp Leu Asn Phe Asn Asp Lys Ser Leu
85 90 95

Trp Ile Lys Asp Thr Ser Thr Asn Gly Thr His Leu Asn Asn Ser Arg
100 105 110

Leu Val Lys Gly Ser Asn Tyr Leu Leu Asn Gln Gly Asp Glu Ile Ala
115 120 125

Val Gly Val Gly Arg Asp Glu Asp Val Val Arg Phe Val Val Val Phe
130 135 140

Gly Asp Lys Tyr Asn Pro Ala Lys Leu Pro Asp Ser Thr Asn Thr Ile
145 150 155 160

Lys Asp Glu Gly Ile Tyr Lys Asp Phe Ile Val Lys Asn Glu Thr Ile
165 170 175

Gly Gln Gly Ala Phe Ala Thr Val Lys Lys Ala Ile Glu Arg Ser Thr
180 185 190

Gly Glu Ser Tyr Ala Val Lys Ile Ile Asn Arg Arg Lys Ala Leu Asn
195 200 205

Thr Gly Gly Gly Ser Ala Met Ala Gly Val Asp Arg Glu Leu Ser Ile
210 215 220

Leu Glu Arg Leu Asn His Pro Asn Ile Val Ala Leu Lys Ala Phe Tyr
225 230 235 240

Glu Asp Met Asp Asn Tyr Tyr Ile Val Met Glu Leu Val Pro Gly Gly
245 250 255

Asp Leu Met Asp Phe Val Ala Ala Asn Gly Ala Ile Gly Glu Asp Ala
260 265 270

Thr Gln Val Ile Thr Lys Gln Ile Leu Glu Gly Ile Ala Tyr Val His
275 280 285

Asn Leu Gly Ile Ser His Arg Asp Leu Lys Pro Asp Asn Ile Leu Ile
290 295 300

Met Gln Asp Asp Pro Ile Leu Val Lys Ile Thr Asp Phe Gly Leu Ala
305 310 315 320

Lys Phe Ser Asp Asn Ser Thr Phe Met Lys Thr Phe Cys Gly Thr Leu
325 330 335

Ala Tyr Val Ala Pro Glu Val Ile Thr Gly Lys Tyr Gly Ser Ser Gln
340 345 350

Met Glu Ser Gln Gln Lys Asp Asn Tyr Ser Ser Leu Val Asp Ile Trp
355 360 365

Ser Leu Gly Cys Leu Val Tyr Val Leu Leu Thr Ser His Leu Pro Phe
370 375 380

Asn Gly Lys Asn Gln Gln Gln Met Phe Ala Lys Ile Lys Arg Gly Glu
385 390 395 400

Phe His Glu Ala Pro Leu Asn Ser Tyr Asp Ile Ser Glu Asp Gly Arg
405 410 415

Asp Phe Leu Gln Cys Cys Leu Gln Val Asn Pro Lys Leu Arg Met Thr
420 425 430

Ala Ala Glu Ala Leu Lys His Lys Trp Leu Gln Asp Leu Tyr Glu Glu
435 440 445

Asp Ser Val Lys Ser Leu Ser Leu Ser Gln Ser Gln Ser Gln Gln Ser
450 455 460

Arg Lys Ile Asp Asn Gly Ile His Ile Glu Ser Leu Ser Lys Ile Asp
465 470 475 480

Glu Asp Val Met Leu Arg Pro Leu Asp Ser Glu Arg Asn Arg Lys Ser
485 490 495

Ser Lys Gln Gln Asp Phe Lys Val Pro Lys Arg Val Ile Pro Leu Ser
500 505 510

Gln His Pro Ala Thr Pro Leu Pro Met Ser Gln Pro Lys Lys Arg Pro
515 520 525

Tyr Gln Ile Asp Pro Arg Thr Asn Lys Lys Val Asp Leu Glu Glu Pro
530 535 540

Ser Thr Ser Lys Lys Val Lys Leu Ser Asp Ser Val Val Ala Glu Asp
545 550 555 560

Tyr Leu Lys Leu Gly Pro Leu Ala Asn Ser Leu Phe Gln Glu Thr Ile
565 570 575

Asn Ile Ser Lys Ser Pro Phe Ser Phe Gly Arg Asn Asp Thr Cys Asp
580 585 590

Cys Glu Ile Asp Asp Asp Arg Leu Ser Lys Leu His Cys Val Ile Thr
595 600 605

Lys Glu Asn Asp Ser Ile Trp Leu Leu Asp Lys Ser Thr Asn Ser Cys
610 615 620

Leu Val Asn Asn Thr Ser Val Gly Lys Gly Asn Lys Val Leu Leu Arg
625 630 635 640

Gly Gly Glu Ile Leu His Leu Phe Phe Asp Pro Leu Ser Ser Gln His
645 650 655

Ile Gly Phe Lys Val Val Leu Val Asp Gln Ser Ser Gly Glu His Lys
660 665 670

Ser Gln Val Glu Val Leu Lys Gln Thr Ser Glu Glu Met Asn Ile Ile
675 680 685

Pro Leu Ile Ser Gly Leu Ser Ser Ile Ser Ser
690 695

<210> 13
<211> 295
<212> PRT
<213> Candida albicans

<400> 13
Met Gly Thr Ser Thr Ser Glu Ala Leu Lys Asn Ile Lys Asn Lys Gln
1 5 10 15

Arg Arg Gln Lys Val Phe Ala Glu Ile Lys His Glu Lys Asn Lys Gln
20 25 30

Arg His Lys Gln Arg Ala Glu Arg Ala Lys Glu Glu Arg Glu Asn Pro
35 40 45

Glu Leu Arg Glu Glu Arg Ile Ala Ala Asn Ile Pro Asp Thr Ile Asp
50 55 60

Ser Lys Arg Ile Tyr Asp Glu Thr Ile Ala Ala Glu Val Glu Gly Asp
65 70 75 80

Asp Glu Phe Gln Ser Tyr Phe Thr Asn Leu Leu Glu Glu Pro Lys Ile
85 90 95

Leu Leu Thr Thr Ser Ala Asn Ala Lys Lys Pro Ala Tyr Glu Phe Ala
100 105 110

Asp Met Ile Met Asp Phe Leu Pro Asn Val Thr Phe Ile Lys Arg Lys
115 120 125

Lys Glu Tyr Thr Met Gln Asp Met Ala Lys Tyr Cys Ser Asn Arg Asp
130 135 140

Phe Thr Ala Leu Leu Val Ile Asn Glu Asp Lys Lys Lys Val Asn Gly
145 150 155 160

Ile Thr Leu Ile Asn Leu Pro Glu Gly Pro Thr Phe Tyr Phe Ser Ile
165 170 175

Thr Ser Ile Val Asp Gly Lys Arg Ile Lys Gly His Gly Lys Ala Gly
180 185 190

Asp Tyr Leu Pro Glu Ile Val Leu Asn Asn Phe Asn Ser Arg Leu Gly

195 200 205

Lys Thr Val Gly Arg Leu Phe Gln Ser Ile Phe Pro His Lys Pro Glu
210 215 220

Leu Gln Gly Arg Gln Val Ile Thr Leu His Asn Gln Arg Asp Tyr Ile
225 230 235 240

Phe Phe Arg Arg His Arg Tyr Ile Phe Arg Asn Glu Glu Lys Val Gly
245 250 255

Leu Gln Glu Gly Pro Gln Phe Thr Leu Lys Leu Arg Arg Met Gln Lys
260 265 270

Gly Val Arg Gly Asp Val Val Trp Glu His Arg Pro Asp Met Glu Arg
275 280 285

Asp Lys Lys Lys Phe Tyr Leu
290 295

<210> 14
<211> 55
<212> PRT
<213> Candida albicans

<400> 14
Met Asn Ser Glu Lys Ile Ile Glu Val Ile Ile Ala Ile Phe Leu Pro
1 5 10 15

Pro Val Ala Val Phe Met Lys Cys Gly Ala Thr Thr Pro Leu Trp Ile
20 25 30

Asn Leu Val Leu Cys Ile Phe Ile Trp Phe Pro Ala Ile Leu His Ala
35 40 45

Leu Tyr Val Val Leu Lys Asp
50 55

<210> 15
<211> 773
<212> PRT
<213> Candida albicans

<400> 15
Met Thr Leu Gly Phe Asp Lys Phe Ile Ser Lys Val Ser Thr His Arg
1 5 10 15

Arg Gln Ser Glu Pro Ser Ile Leu Glu Ile Ala Ala Thr Asn Ser Gln
20 25 30

Asn Lys Ser Arg Arg Leu Ser Met Asp Asn Gly His Cys Tyr Val Arg
35 40 45

Glu Ser Thr Asn Asn His His His Leu Asn Thr Val Val Asp Asn Leu
50 55 60

Arg Gln Arg Ala Gly Ser Phe Ser Phe Ile Ser His His His Asn His
65 70 75 80

His Gln Asn Ser His Asp Asn Tyr Thr Val Asp Pro Leu Thr Ser Asn
85 90 95

Gly Ala Arg Ile Ser Arg Ser Arg Ser Arg Ser Lys Ser Val Gly His
100 105 110

Gly Glu Ala Ile Ser Pro Ala Tyr Phe Ser Lys Asn Lys Thr Lys Asp
115 120 125

Leu Val Lys Gln Glu Thr Ala His Ile Ile Ser Lys Lys Leu Leu Asn
130 135 140

Met Leu Gln Asp Leu Asp Leu Gln Asn Pro Ile Ala Leu Lys Thr Ile
145 150 155 160

Ser Gln Gly Ser Glu Ser Lys Phe Cys Lys Ile Tyr Val Ser Asn Thr
165 170 175

Asn Asn Cys Ile Tyr Leu Pro Ala Ala Ser Ser Thr Ser Phe Thr Tyr
180 185 190

Glu Asp Asp Glu Asn Gly Gly Val Ile Ile Ala Glu Asp Arg Asn Asp
195 200 205

Glu Met Pro Thr Ala Val Asn Asn Asn Thr Leu Ser Met Asp Ser Ile
210 215 220

Asn His Ser Glu Thr Asp Phe Ser Asp Ser Pro Pro Pro Pro Asp Leu
225 230 235 240

Phe Ser Lys Met Lys Ser Phe His Ser Pro Asn Tyr Leu Thr Ser Lys
245 250 255

Ile Asp Ser Glu Cys Pro Ile Pro His Thr Phe Ala Val Ile Val Glu
260 265 270

Leu Thr Lys Asp Ser Leu Ile Ile Lys Asp Leu His Phe Gln Phe Gln
        275   280   285

Ser Leu Thr Thr Ile Leu Trp Pro Thr Gly Asp Ala Tyr Asn Arg Thr
    290   295   300

His Ala Lys Glu Lys Phe Thr Ile Gly Asn Met Glu Trp Arg Thr Ser
305   310   315   320

Leu Ser Asp Ala Asp Tyr Tyr Ile Asn Ser Ser Asn Ser Asn Asp Val
                325   330   335

Lys Ser Lys Asn Leu Gly Pro Glu Asp Leu Ile Asn Arg Thr Arg Glu
            340   345   350

Tyr Lys Leu Ile Asp Ile Glu Glu Pro Asn Asn Ser Ser Asn Ser Leu
        355   360   365

Ser Asp Asp Asp Met Asp Ile Asn Asn Ile Thr Ser Pro Leu Ser Thr
    370   375   380

Ser Pro Thr Ser Ser Ser Thr Ser Thr Asn Ser Thr Ser Asn Ser Leu
385   390   395   400

Gly Ser Asp Ser Tyr Lys Ala Gly Leu Tyr Val Phe Leu Leu Pro Ile
                405   410   415

Leu Leu Pro Glu His Ile Pro Ala Ser Ile Val Ser Ile Asn Gly Ser
            420   425   430

Leu Ala His Thr Leu Ser Val Glu Cys Asn Lys Tyr Thr Asp Lys Leu
        435   440   445

Asn Arg Lys Ser Lys Val Ser Ala Ser Tyr Asn Leu Pro Met Val Arg
    450   455   460

Thr Pro Pro Asn Ile Gly Asn Ser Ile Ala Asp Lys Pro Ile Tyr Val
465   470   475   480

Asn Arg Ile Trp Asn Asp Ala Val His Tyr Ile Ile Thr Phe Pro Arg
                485   490   495

Lys Tyr Val Thr Leu Gly Cys Glu His Met Ile Asn Val Lys Leu Ser
            500   505   510

Pro Met Val Lys Asp Val Val Ile Lys Arg Ile Lys Phe Asn Val Leu
        515   520   525

Glu Arg Ile Thr Tyr Val Ser Lys Asn Leu Ser Arg Glu Tyr Asp Tyr
530 535 540

Asp Ser Glu Asp Pro Tyr Cys Ile His Pro Val Ser Lys Glu Asn Lys
545 550 555 560

Val Arg Glu Arg Val Val Ser Leu Tyr Glu Leu Lys Thr Lys Ala Lys
565 570 575

Gln Ser Ser Gly Gly His Leu Glu Ala Tyr Lys Gln Glu Val Met Lys
580 585 590

Cys Pro Glu Asn Asn Leu Leu Phe Ser Cys Tyr Glu Val Glu Asn Asp
595 600 605

Asn Asn Asn Gly Asn Gly Asn Gly Asn Gly Asn Gly Asn Lys Asn Val
610 615 620

Lys Gln Lys Asn Lys Asp Gln Pro Met Ile Ala Thr Pro Leu Asp Ile
625 630 635 640

Asn Val Ser Leu Pro Phe Leu Thr Thr Met Ser Asp Ser Leu Ile Met
645 650 655

Thr Ser Ala Ile Glu Glu Glu Gly Ser Asp Ser Pro His Thr Ser Arg
660 665 670

Arg Gly Ser Ala Val Ser Met Thr Asp Asn Asn Thr Thr Pro Ser Asn
675 680 685

Asn Asn Pro Leu Ser Pro Phe Leu Gly Ala Val Glu Thr Asn Gly Ala
690 695 700

Ser Ile Asn Glu Ile Gly Asp His Thr Leu Phe Pro Asp Ser Asn Phe
705 710 715 720

Arg His Ile Glu Ile Lys His Arg Leu Gln Val Thr Phe Arg Ile Ser
725 730 735

Lys Pro Asp Ser Asp Asn Lys Met His His Tyr Glu Val Val Ile Asp
740 745 750

Thr Pro Ile Val Leu Leu Ser Ser Lys Cys Gln Glu Asp Ser Pro Pro
755 760 765

Pro Tyr Ser Ser Val
770

<210> 16
<211> 90
<212> PRT
<213> Candida albicans

<400> 16

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Met Gly Glu Gly Thr Pro Ser Leu Gly Lys Arg His Asn Lys Ser His
1               5                   10                  15

Thr Leu Cys Asn Arg Cys Gly Arg Arg Ser Phe His Val Gln Lys Lys
            20                  25                  30

Thr Cys Ser Ser Cys Gly Tyr Pro Ala Ala Lys Met Arg Ser His Asn
        35                  40                  45

Trp Ala Leu Lys Ala Lys Arg Arg Arg Thr Thr Gly Thr Gly Arg Met
    50                  55                  60

Ala Tyr Leu Lys His Val Thr Arg Arg Phe Lys Asn Gly Phe Gln Thr
65                  70                  75                  80

Gly Val Ala Lys Ala Gln Thr Pro Ser Ala
                85                  90
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